Addressing the Burden of Antimicrobial Resistance in Vietnamese Hospitals

Thesis

ADDRESSING THE BURDEN OF ANTIMICROBIAL RESISTANCE IN VIETNAMESE HOSPITALS

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Abstract

Hospital acquired infections (HAIs), especially ventilator associated respiratory infection (VARI) cause significant morbidity and mortality, and disproportionally so in low- and middle-income countries (LMICs), including Vietnam, where infection control in hospitals is often neglected. The management of HAIs in these settings is challenging because of the high proportions of antimicrobial drug resistance and limitations of laboratory diagnostics, financial and human resources in terms of knowledge and skills for antimicrobial stewardship and infection prevention and control.

Because resistance is driven by use of antimicrobials, my thesis started with a question on use and cost of antimicrobials in public hospitals in the country followed by a detailed assessment of use and cost of antimicrobials in the management of ventilator associated respiratory infections (VARI). I obtained detailed bids from hospitals and provincial departments of health representing 28.7% (1.68 / 5.85 billion US$) of the total hospital medication budget in Vietnam. Antimicrobials represented 28.6% of these costs. Antimicrobials were stratified using the Access, Watch, Reserve (AWaRe) groups proposed by WHO in 2017. I showed that the most commonly used antimicrobials across sites were second generation cephalosporins (20.3% of total procured defined daily dose, DDD) followed by combinations of penicillins and beta-lactamase inhibitors (18.4% of total procured DDD). The most expensive antimicrobials are the last resort antimicrobials, which can considerably increase the cost of treatment for patients with HAIs caused by multidrug resistant pathogens in critical care units in Vietnam. In recognition of this problem, I estimated the excess cost of management of VARI using a costing model study. At the current incidence rate of 21.7 episodes per 1000 ventilation-days, I estimated there were 34,428 episodes of VARI nationally, associated with a direct cost of more than US$ 40 million per year. Our studies showed the need for an affordable and scalable intervention in critical care units to reduce the burden of VARI and provide cost savings for national health expenditure.

My studies also showed that antimicrobial costs are a major component of the excess cost of VARI management in Vietnam (51.1%) and that a one day reduction in the duration of antimicrobial therapy can save US$ 1.72 million. Therefore, my thesis has focused on interventions to prevent VARI and to shorten antimicrobial therapy. In recognition of

human resources constraints in Vietnam, including for microbiology diagnostics and critical care nursing, I have studied automatic technology and equipment, including matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) for rapid identification of pathogens and continuous automatic cuff pressure control device to prevent VARI. To examine effectiveness of these intervention, I conducted 2 randomised controlled trials to evaluate the clinical effectiveness of matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) in optimizing antimicrobial therapy and to evaluate the effectiveness of continuous cuff pressure control in preventing VARI. For the latter, pending unblinding and final results I describe the implementation of the trial and report the incidence of hospital acquired bloodstream infection during this trial.

A diagnostic randomised controlled trial (RCT) was conducted to evaluate the impact of MALDITOF-MS versus conventional diagnostics in improving antimicrobial use in patients with confirmed infection. Although MALDITOF-MS provided more rapid identification of invasive bacterial and fungal pathogens than conventional microbiology, the proportion of patients on optimal therapy at 24 or 48 hours after growth of specimen did not increase. These findings showed that without human resources and an effective antimicrobial stewardship programme, technology alone cannot provide a solution for antimicrobial overuse in hospitals in LMICs.

A randomized controlled clinical trial was conducted to evaluate the effectiveness of continuous cuff pressure control versus daily manual cuff measurement (VARI-prevent). In this study I recruited and followed-up 597 adult patients who were admitted to ICUs and were intubated within 48 hours of admission. The patients were randomised to receive either continuous or manual cuff pressure measurement and control and were followed for occurrence of VARI during ICU stay and up to 90 days after randomisation. The study has completed recruitment and follow-up and final analysis is ongoing. The overall rate of VARI and VAP in eligible patients was 23.7% (140/591) and 17.3% (102/591) respectively. The data from this trial (VARI-prevent) was analysed to estimate the incidence density rate of hospital acquired bloodstream infection (HABSI) in 3 ICUs in Vietnam for the first time. The most common pathogens causing HABSI were *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Coagulase-Negative staphylococci. Polymicrobial culture results were reported in 6.8% (3/44) patients with

culture confirmed HABSI. The rate of HABSI and central line associated BSI (CLABSI) were 7.4% (44/591) and 9.3% (31/333), respectively. The incidence density rate of HABSI and CLABSI were 3.76 per 1000 patients-days and 8.43 per 1000 catheter-days, respectively. This suggests that the implementation of infection prevention and control bundle including catheter care is important to reduce the high incidence of HABSI in Vietnam.

The findings in my thesis are relevant to healthcare professionals and policy stakeholders. It demonstrates the magnitude of HAI burden and creates awareness of potential beneficial interventions. Results of my trials will be helpful to inform decisions to establish the antimicrobial stewardship programmes and infection prevention and control bundles to improve patients' outcomes.

Acknowledgement

I'm grateful to the Oxford University Clinical Research Unit (OUCRU) for my PhD scholarship. This is a special programme for young researchers to obtain thorough knowledge of conducting and managing clinical trials whilst receiving effective supervision from experienced supervisors.

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Publications

Directly relating to the work in this thesis

1. **Dat VQ**, Nadjm B, Campbell JI, Dung VTV, Torre A, Tu NTC, Van NTT, Trinh DT, Lan NPH, Trung NV, Hang NTT, Hoi LT, Baker S, Wolbers M, Chau NVV, Van Kinh N, Thwaites GE, van Doorn HR, Wertheim HFL. 2019. A randomised controlled trial of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) versus conventional microbiological methods for identifying pathogens: Impact on optimal antimicrobial therapy of invasive bacterial and fungal infections in Vietnam. J Infect doi:10.1016/j.jinf.2019.03.010.
2. **Dat VQ**, Geskus RB, Wolbers M, Loan HT, Yen LM, Binh NT, Chien LT, Mai NTH, Phu NH, Lan NPH, Hao NV, Long HB, Thuy TP, Kinh NV, Trung NV, Phu VD, Cap NT, Trinh DT, Campbell J, Kestelyn E, Wertheim HFL, Wyncoll D, Thwaites GE, van Doorn HR, Thwaites CL, Nadjm B. 2018. Continuous versus intermittent endotracheal cuff pressure control for the prevention of ventilator-associated respiratory infections in Vietnam: study protocol for a randomised controlled trial. Trials 19:217.
3. **Dat VQ**, Huong VTL, Turner HC, Thwaites L, van Doorn HR, Nadjm B. 2018. Excess direct hospital cost of treating adult patients with ventilator associated respiratory infection (VARI) in Vietnam. PLoS One 13:e0206760.
4. **Dat VQ**, Vu HN, Nguyen The H, Nguyen HT, Hoang LB, Vu Tien Viet D, et al. Bacterial bloodstream infections in a tertiary infectious diseases hospital in Northern Vietnam: aetiology, drug resistance, and treatment outcome. BMC infectious diseases 2017; 17(1):493.
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7. Li R, van Doorn HR, Wertheim HF, Khue LN, Ha NT, **Dat VQ**, Hanh CT, Nga DT, Trang NN, Nadjm B, Cluzeau F, Kinh NV, Trung NV, Huong NT, Chau NQ, Huong Q, Thao LT, Hong LT, Oanh TT, Islam J, Roberts CM, Chalkidou K. 2016. Combating antimicrobial resistance: quality standards for prescribing for respiratory infections in Vietnam. Lancet Glob Health 4:e789.

Declaration

I confirm that the majority of the work presented in this thesis is my own and was conducted under the supervision of Drs. Behzad Nadjm, C. Louise Thwaites and H. Rogier van Doorn at the Oxford University Clinical Research Unit in Hanoi, Vietnam and with valuable assistance from OUCRU's Clinical Trial Unit and the collaborating hospitals (National Hospital for Tropical Diseases (NHTD) in Hanoi and Hospital for Tropical Diseases (HTD) and Trung Vuong Hospital (TVH) in Ho Chi Minh city) in data collection and management of studies. I received support for statistical analysis from Prof. Ronald B Geskus, Mr. Vu Tien Viet Dung and Dr. Marc Choisy from OUCRU. During my PhD programme, I attended extensive training courses on continuing professional developments from OUCRU's training department.

The following work has been published in international peer-reviewed open access journals: the section 2.2 in Chapter 2 in Trials in 2018, the work in Chapter 4 in PLoS One in 2018 and the work in Chapter 5 in the Journal of Infection in 2019. The work presented in Chapter 5 was co-authored by myself and Dr. Behzad Nadjm and Prof. Heiman F. L. Wertheim was the director of the project. This thesis has not been submitted for a degree or other qualification to any other universities.

Abbreviations

Abbreviation	Words and phrase
3GC	Third-generation cephalosporin
ABC	Active Bacterial Core Surveillance
AMR	Antimicrobial resistance
AOR	Adjusted odd ratio
API	Analytical profile index
ASP	Antimicrobial stewardship program
BSI	Bloodstream infection
CARB	Carbapenem
CCU	Critical care unit
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
CI	Confidence interval
CLABSI	Central line associated bloodstream infection
CoNS	Coagulase-negative *staphylococci*
CPC	Continuous endotracheal cuff pressure control
CPE	Carbapenemase-producing Enterobacteriaceae
CRF	Case record form
DDD	Defined daily dose
DNA	Deoxyribonucleic acid
ECDC	European Centre of Disease Prevention and Control
ESBL	Extended spectrum β-lactamase
ETT	Endotracheal tube
GNI	Gross national income
HABSI	Hospital acquired bloodstream infection
HAI	Hospital acquired infection
HAP	Hospital acquired pneumonia
HIC	High-income country
HTD	Hospital for Tropical Diseases
ICU	Intensive care unit
IQR	Interquartile range
LMIC	Low- and middle-income country
MALDITOF-MS	Matrix assisted laser desorption ionization-time of flight mass spectrometry

Abbreviation	Words and phrase
MDR	Multidrug resistance
MRSA	Methicillin-resistant Staphylococcus aureus
NDM-1	New Delhi Metallo-β-lactamase-1
NHSN	National Healthcare Safety Network
NHTD	National Hospital for Tropical Diseases
NNIS	National Nosocomial Infections Surveillance System
OAT	Optimal antimicrobial treatment
OR	Odd ratio
RCT	Randomised control trial
SAE	Serious adverse event
SEA	South-east Asia
SSI	Surgical site infection
TVH	Trung Vuong Hospital
UK	United Kingdom
UMIC	Upper-middle-income country
USA	United States of America
USD	United States dollar
UTI	Urinary tract infection
VAP	Ventilator associated pneumonia
VARI	Ventilator associated respiratory infection
VAT	Ventilator associated tracheobronchitis
VND	Vietnamese Dong
VRE	Vancomycin-resistant Enterococcus
VRSA	Vancomycin-resistant Staphylococcus aureus
WHO	World Health Organisation

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Chapter 1: **General introduction**

1.1. The global antimicrobial resistance threat

1.1.1. The role of the hospital and ICUs in the cycle of antibiotic resistance

The discovery of antibiotics is arguably the greatest medical achievement in the history of mankind (Ferriman, 2007). Since their introduction into patient care, antibiotics have saved millions of lives and enabled advances in many other fields of medicine, including critical care, surgery, transplantation and cancer treatment (Spellberg et al., 2011). However, we are now at the dawn of the post-antibiotic era where the continued effectiveness of antibiotics is waning along with an estimated global loss of 10 million lives a year and 100 trillion USD by 2050 due to antibiotic resistance (O'Neill, 2014).

Antibiotics are classified as original natural compounds produced by fungi and saprophytic bacteria or derived or chemically synthesized from natural compounds (Demain and Sanchez, 2009). The emergence and spread of antibiotic resistance reflects an ancient, natural phenomenon of antibiotic resistance gene distribution (D'Costa et al., 2011) and a classic Darwinian selection process accelerated by the widespread mass use of antibacterial drugs by humans in healthcare and food production.

The terms overuse, misuse and inappropriate use are applied for antibiotic prescription without or with minimal benefit to patients (Smith et al., 2018). Global antibiotic resistance is affected by multiple ecological and social drivers in different environments, including use (appropriate or inappropriate) in human healthcare settings, but also use in animals for food production in the agricultural and aquacultural environments – representing the majority of use globally (Andersson and Hughes, 2014, Centers for Disease Control and Prevention (CDC), 2013a).

Hospitals, particularly intensive care units (ICUs) present a conducive environment for emergence, spread and selection of antimicrobial resistance (AMR) (Figure 1-1). High selective pressure favouring resistant bacteria is exerted by the intensive and frequent antibiotic use in the hospital environment (Fortin et al., 2014), whilst poor quality infection control programmes and other conditions such as a high density of extremely vulnerable population with immunocompromised conditions enable transmission of these resistant

bacteria (Nicolle and World Health Organization. Anti-Infective Drug Resistance Surveillance and Containment Team., 2001). Therefore, hospitals act as amplification vessels for resistance genes and resistant pathogens, which may continue to spread to the community. Indeed, infections acquired within the hospital setting (hospital acquired infections) are generally associated with bacteria that are resistant to more antibiotics than infections acquired in the community.

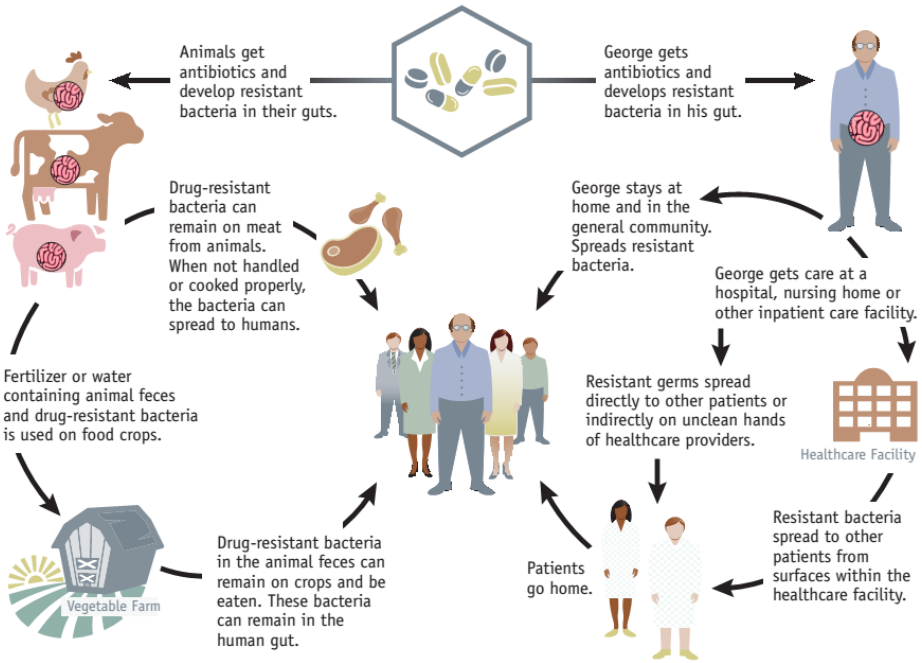


Figure 1-1. The cycle of antibiotic resistance (Centers for Disease Control and Prevention (CDC), 2013a).

Among hospitalised patients, those in ICUs receive more broad-spectrum antibiotics at prolonged treatment durations, and undergo more invasive medical procedures putting them at higher risk of infection with drug resistant bacteria (Voor In 't Holt et al., 2014). Currently, carbapenem resistant Gram-negative bacteria, especially Enterobacteriaceae, are recognised as the greatest threat to patient safety in hospitals (European Centre for Disease Prevention and Control, 2019). In a systematic review and meta-analysis of studies published between 1987 and 2012, important significant risk factors for carbapenem-resistant *Pseudomonas aeruginosa* infection were prior administration of carbapenems (OR = 7.09, 95% CI 5.43–9.25), medical devices (OR = 5.11, 95%CI 3.55–7.37), and ICU admission (OR= 3.02, 95%CI=1.62–5.61). Other statistical significant factors were other antibiotic use, underlying diseases and length of hospital stay (Voor In 't Holt et al., 2014).

The US National Nosocomial Infections Surveillance showed that in 1996 among 262 hospitals higher rates of antibiotic use corresponded to higher antibiotic resistance among *Escherichia coli* for ceftriaxone, *P. aeruginosa* for ceftazidime and piperacillin/tazobactam and enterococci for vancomycin in ICUs in comparison with non-ICU settings (Fridkin et al., 1999). The mean proportion of resistance among selected important pathogen-antimicrobial pairings was highest among ICU patients (21%), followed by non-ICU inpatients (17%) and lowest among outpatients (12%) (Zhang et al., 2006). In a study conducted between 2011 and 2013 in Europe and North America, susceptibility of *Enterobacteriaceae* was lower in ICUs than non-ICU wards for all antibiotics tested using the Clinical and Laboratory Standards Institute (CLSI) guidelines (MDR rate was 16.4% in ICU vs 11.4% in non-ICU) ($P < 0.05$) in 18 European countries and in Canada and the USA (except for ciprofloxacin and levofloxacin) (Lob et al., 2015).

Hospitals play an important role in persistence and spread of antimicrobial resistance (Figure 1-2). On dry surfaces, most nosocomial bacteria can survive from days to months (4-5 months for *Acinetobacter* spp and *Enterococcus* spp, 16 months for *E. coli* and *P. aeruginosa* and more than 30 months for *Klebsiella* spp) (Kramer et al., 2006). Exposure to antibiotics will favour the survival of resistant isolates over their susceptible counterparts. This can increase the risk for acquisition of a resistant pathogen for a newly hospitalised patient from the prior room occupant (Mitchell et al., 2015). Additionally, many hospital pathogens can produce biofilms during their colonisation of surfaces, facilitating prolonged survival, colonisation of medical devices, and subsequent device-related infections (Donlan and Costerton, 2002, Donlan, 2001). The hospital water environment can contribute to the chain of transmission by serving as a reservoir of nosocomial pathogens. Water was reported as an important source of nosocomial outbreaks in the USA, associated with nearly 1400 deaths from *P. aeruginosa* pneumonia alone each year (Anaissie et al., 2002). Carbapenemase- or extended-spectrum β-lactamase-producing *Enterobacteriaceae* can contaminate sinks in hospital and cause transmission between sinks, to healthcare worker's hands and in turn to patients in ICUs (Lowe et al., 2012, De Geyter et al., 2017, Snitkin et al., 2012, Clarivet et al., 2016).

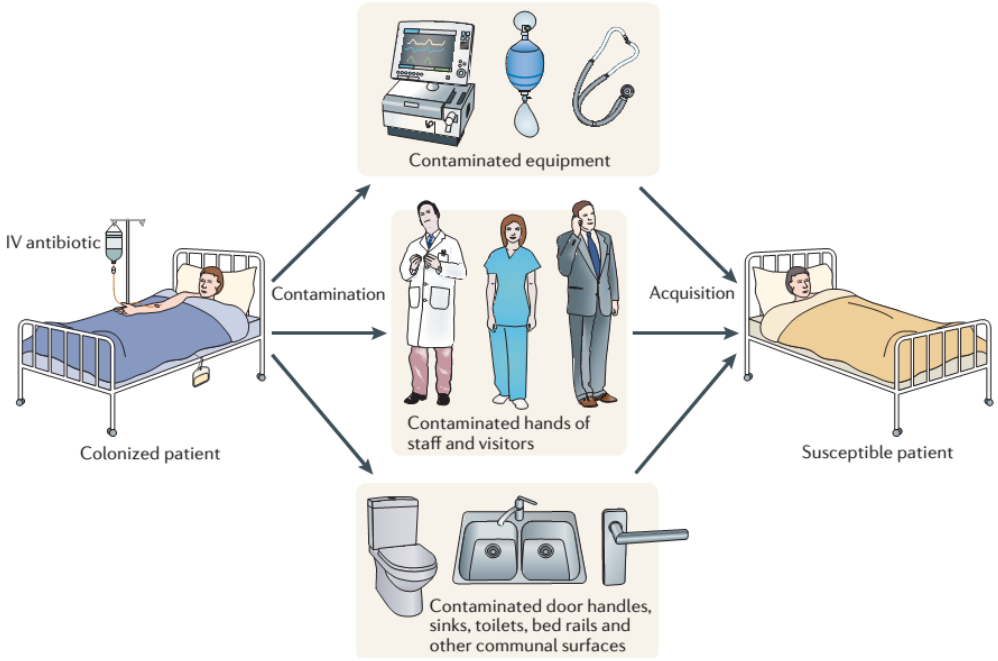


Figure 1-2. Major route of transmission of nosocomial antimicrobial resistant pathogens in hospitals.

Adapted from Arias et al (Arias and Murray, 2012).

In many low- and middle-income countries (LMIC), antimicrobial drug resistance poses an increasing burden on already stretched healthcare systems (Okeke et al., 2005), and the proportion of resistant bacteria is growing more rapidly in these countries. In a study of antibiotic resistance across 3 countries in different geographic and economic zones, the proportion and increase of resistant pathogens was highest in China (22% increase from 1994 to 2000, data from China's National Center for Antimicrobial Resistance), followed by Kuwait (17% increase from 1999 to 2003, data from a single large teaching hospital, Mubarak Al-Kabeer Hospital,) and the USA (6% increase from 1999 to 2002, data from National Nosocomial Infections Surveillance System (NNIS) for hospital-based resistance and the U.S. Active Bacterial Core Surveillance (ABC)) (Zhang et al., 2006). Many factors can contribute to the increase of drug resistance in the hospitals in LMICs, including (inappropriate) use of antibiotics, lack of knowledge about infectious diseases and microbiology, lack of diagnostics, understaffing, overcrowded hospitals, lack of effective infection prevention and control and in some the growing phenomenon of medical tourism (Okeke et al., 2005, World Health Organization. Anti-Infective Drug Resistance Surveillance and Containment Team., 2001, Hsu et al., 2017, McNulty et al., 2018).

1.1.2. Aetiology and drug resistance profile of common pathogens causing hospital acquired infections

Many microorganisms can cause nosocomial (hospital acquired) infections, including different viruses, bacteria, fungi and parasites. Bacteria are the most common causative pathogens, and *E. coli* and the ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter spp*) are the most important species associated with hospital acquired infections in the world (Boucher et al., 2009). In 2013, the United States of America's Centers for Disease Control and Prevention (US CDC) raised awareness of antibiotic resistance threats that required urgent actions to respond. They described 18 priority bacteria-drug combinations in both health care and community settings focusing on incidence, clinical and economic impacts, availability of current treatment and prevention (Centers for Disease Control and Prevention (CDC), 2013a). These pathogens were categorized into one of three levels of antibiotic resistance: urgent, serious and concerning. carbapenemase-producing *Enterobacteriaceae* (CPE) were assigned to the urgent category along with *Clostridium difficile* and cephalosporin resistant *Neisseria gonorrhoeae* (Table 1-1).

Table 1-1. The US CDC's priority pathogens list of antibiotic-resistant bacteria (Centers for Disease Control and Prevention (CDC), 2013a)

Antibiotic Resistance Threats

Threat Level of Urgent

- ***Clostridium difficile***
- **Carbapenem-resistant *Enterobacteriaceae***
- Drug-resistant *Neisseria gonorrhoeae*

Threat Level of Serious

- **Multidrug-resistant *Acinetobacter*** (resistant to three or more drug classes)
- Drug-resistant *Campylobacter*
- **Fluconazole-resistant *Candida***
- **Extended spectrum β-lactamase producing *Enterobacteriaceae* (ESBLs)**
- **Vancomycin-resistant *Enterococcus* (VRE)**
- **Multidrug-resistant *Pseudomonas aeruginosa*** (three or more drug classes)
- Drug-resistant non-typhoidal *Salmonella*
- Drug-resistant *Salmonella* Typhi
- Drug-resistant *Shigella*
- **Methicillin-resistant *Staphylococcus aureus* (MRSA)**
- Drug-resistant *Streptococcus pneumoniae*
- Drug-resistant tuberculosis

Threat Level of Concerning

- **Vancomycin-resistant *Staphylococcus aureus* (VRSA)**
- Erythromycin-resistant Group A *Streptococcus*
- Clindamycin-resistant Group B *Streptococcus*

Nosocomial pathogens are in bold.

In 2017, WHO released a global priority pathogens list of 12 pairings of bacteria and antibiotic resistance phenotypes for research and development of new treatments and stratified these into three priority levels as critical, high and medium (World Health Organization, 2017a). The critical group includes nosocomial multidrug resistant bacteria: carbapenem-resistant *A. baumannii*, carbapenem-resistant *P. aeruginosa* and carbapenem/3rd generation cephalosporin-resistant *Enterobacteriaceae* (see Table 1-2).

Table 1-2. The WHO's priority list of antibiotic-resistant bacteria for research and development (R&D) of new and effective antimicrobial treatments (World Health Organization, 2017a)

Antibiotic-resistant microorganism

Priority 1: CRITICAL

- ***Acinetobacter baumannii*, carbapenem-resistant**
- ***Pseudomonas aeruginosa*, carbapenem-resistant**
- ***Enterobacteriaceae*, carbapenem-resistant, ESBL-producing**

Priority 2: HIGH

- ***Enterococcus faecium*, vancomycin-resistant**
- ***Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant**
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

Nosocomial pathogens are in bold.

In 2015 and 2018, WHO released the reports of the Global Antimicrobial Resistance Surveillance System (GLASS) for selected priority specimen and pathogens (see Table 1-3) (Organization, 2015, World Health Organization, 2018a). This part of the thesis will focus on the critical group of prioritized bacteria defined by the WHO's priority pathogens list as all of these represent enormous challenges in Vietnamese hospitals.

1.1.2.1. *Acinetobacter baumannii*

Acinetobacter are Gram-negative, strictly aerobic coccobacilli, and the genus comprises 17 named species and 15 genomic species (gen. sp.) designated by DNA–DNA hybridization, among which *Acinetobacter baumannii* is the most clinically important species (Dijkshoorn et al., 2007).

Table 1-3. WHO priority specimens and pathogens for surveillance of AMR

Specimen	Case definition	Prioritized pathogens for surveillance
Blood	Isolation of pathogen from blood	*E. coli* *K. pneumoniae* *A. baumannii* *S. aureus* *S. pneumoniae* *Salmonella spp*
Urine	Significant growth in urine specimen	*E. coli* *K. pneumoniae*
Faeces	Isolation of Salmonella spp. or Shigella spp. from stools	*Salmonella spp*. *Shigella spp*.
Urethral and cervical swabs	Isolation of N. gonorrhoeae	*N. gonorrhoeae*

The natural habitat of *Acinetobacter* species consists of soil, water, and vegetables and many members of the genus *Acinetobacter* have been recovered from human skin (Dijkshoorn et al., 2007). However, the proportion of individuals carrying *A. baumannii* on healthy human skin is usually low (0.5%-10.4%) and community *A. baumannii* isolates are often susceptible and unrelated (Seifert et al., 1997, Zeana et al., 2003) whilst the proportion of *A. baumannii* colonisation in hospital ranged from 32.4% to 64% and most hospital isolates are multidrug resistant and closely related (Zeana et al., 2003, Lee et al., 2010). *A. baumannii* can easily contaminate healthcare workers' gowns, gloves, and hands and seems more easily transmitted than other multidrug resistant pathogens such as *P. aeruginosa,* MRSA or VRE (Morgan et al., 2010). *A. baumannii* can cause a spectrum of nosocomial infection in critically ill patients, of which the majority are ventilator-associated respiratory infections with other manifestations including bloodstream infection, catheter-associated urinary-tract, wound infections and meningitis (Peleg et al., 2008). These demonstrate the important role of the *A. baumannii* reservoir and transmission in outbreaks of nosocomial infection.

The resistance of *A. baumannii* to carbapenem has increased globally since 2006 (Peleg et al., 2008). The report from National Healthcare Safety Network (NHSN) in the USA

showed the overall proportion of carbapenem resistance in *A. baumannii* was 33% in 2006–2007 (Hidron et al., 2008). During the period of 2011 to 2014, NHSN reported a higher frequency of resistance among *A. baumannii* strains than in 2006 and with a wide variability among types of infections. In 2014, the prevalence of carbapenem resistance in *A. baumannii* was 64% among isolates causing catheter-associated urinary tract infections, 55% in ventilator-associated pneumonias (VAPs), 46.4% from central line-associated bloodstream Infections, and 33.3% in surgical site infections (SSIs) (Weiner et al., 2016). In the MYSTIC programme in 11 European countries from 1997-2000, among 490 tested isolates, *A. baumannii* was highly susceptible to meropenem (97–100%) and imipenem (93–100%) in all countries except Italy, Turkey and the UK (susceptibility of 66-77% to meropenem and 62-78% to imipenem) (Turner and Greenhalgh, 2003).

The proportion of carbapenem-resistance in clinical isolates of *A. baumannii* in South-east Asia (SEA) may be the highest in the world (Hsu et al., 2017). The SEA region consists 11 countries, including 2 high income economies (Gross national income, GNI per capita of US$ 12,376 or more, including Singapore and Brunei), 2 upper middle income economies (GNI per capita of US$ 3,996 and US $12,375, including Malaysia and Thailand) and 7 lower-middle income economies (GNI per capita between US$ 1,026 and US$ 3,995, including Myanmar, Cambodia, Indonesia, Laos, Philippines, Timor-Leste and Vietnam) (World Bank, 2019). Among upper middle- and high- income SEA countries, where national hospital-based antimicrobial resistance surveillance has been established, the proportion of carbapenem resistance in *A. baumannii* was 61% in Malaysia (2016) (Institute for Medical Research, 2016) and 71% in Thailand (2016) (National Antimicrobial Resistance Surveillance Center, 2016). In Singapore, the proportion of carbapenem resistance in *A. baumannii* was 49.6% in a national survey (Hsu et al., 2007) and it remained unchanged during the period of 2011-2015, in Singapore's largest hospital (Teo et al., 2016) (in the absence of publicly available recent national data). For other SEA countries with low- and lower middle-incomes, in a systematic review from the period 2002-2016, carbapenem resistance among *A. baumannii* isolates was reported as 43%-92% in Vietnam, 50-85% in Indonesia, 54.1% in the Philippines and 5.9% in Cambodia. Data were not published for the remaining countries in the region, including Myanmar, Laos, Brunei and Timor-Leste (Hsu et al., 2017).

Table 1-4. Antimicrobial susceptibility profiles of common nosocomial carbapenem-resistant pathogens in Vietnamese ICUs

Study	Study period	Study setting	Clinical specimen	*A. baumannii*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
(Johansson et al., 2011)	1/2007-3/2008	3 ICU with a total of 62 beds	Blood, respiratory aspirate and urine*	69.1% (105/152)	55.7% (69/124)	2.9% (3/103)	0% (0/61)
(Van et al., 2014)	2009	Single ICU with 15 beds	Blood, respiratory aspirate and pus	92.1% (52/68)			
(Kiratisin et al., 2012)	4-7/2010	3 hospitals*	Isolates from intra-abdominal, bloodstream, and pneumonia infections	89.5%	46.7%		
(Phu et al., 2016)	10/2012-9-2013	15 adult ICUs	Isolates from pneumonia, bloodstream, surgical site and urinary tract infection	89.2%	55.7%	14.9%	
(Le et al., 2016)	10/2012-9-2013	6 paediatric ICUs		67%	71%	55%	42%
(Tada et al., 2013)	2008-2011	2 ICUs	Respiratory, blood and pus	51%	53%		
(Le Minh et al., 2015)	1/2011-6/2012	Single ICU	Tracheal aspirate	84%			
(Biedenbach et al., 2016)	2012–2014	5 hospitals	respiratory tract specimens	92.5% (904)	42.9% (529)		
(Nhu et al., 2014)	11/2012 to 9/ 2013	2 paediatric hospitals		67%	71%	55%	42%
(Tran et al., 2017)	11/2014 – 9/2015	Single ICU	Bronchoalveolar lavage (BAL) fluid	93.2%	86.2%	25.6%	
(Tuan Anh et al., 2017)	2012-2014	3 hospitals	Sputum, pus, blood and fluid aspirates	86.7%			

1.1.2.2. Pseudomonas aeruginosa

P. aeruginosa are non-fermenting Gram-negative bacilli living in soil and aqueous environments (Gellatly and Hancock, 2013). *P. aeruginosa* is not commonly part of the normal human flora, with an estimated 5% of individuals colonised (Kropec et al., 1993). However, high colonisation rates are associated with a risk of severe *P. aeruginosa* infection in patients with long term hospitalisation and/or immunocompromising conditions, such as cystic fibrosis, bronchiectasis, severe burns, hematologic malignancies or critical illness. In a study among 1314 patients in 10 ICUs in France, *P. aeruginosa* colonisation developed in 11-21% of patients admitted to ICU during their stay and the risk factors for *P. aeruginosa* acquisition were increased age, history of previous acquisition, duration of mechanical ventilation for more than 10 days and exposure to non-antipseudomonal antibiotics and contaminated water (Venier et al., 2014). In SEA in 2010, the proportion of carbapenem resistant *P. aeruginosa* among isolates in hospitalised patients was lowest in Singapore (23.3%) and highest in Vietnam (46.7%) (Suwantarat and Carroll, 2016).

P. aeruginosa is resistant to a broad range of antimicrobials through intrinsic, acquired and adaptive mechanisms and its additional swarming motility and biofilm-forming capacity causes difficulty in treating infections (Breidenstein et al., 2011). Carbapenem is only second choice empirical antipseudomonal treatment after ceftazidime, and should not be used as monotherapy because of rapid resistance development. Data from the European Antimicrobial Resistance Surveillance Network (EARS-net) and a global WHO network, including both non-ICU wards and ICUs with a mean number of 5160 isolates per year, showed the proportion of carbapenem resistance in *P. aeruginosa* has increased from 6.8 to 28.8% during 1996–2015 (Karampatakis et al., 2018).

1.1.2.3. Enterobacteriaceae

Enterobacteriaceae are a large family of important Gram-negative bacilli that are part of the normal flora in the human gastrointestinal tract and include multiple genera among which *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, and *Citrobacter* are most frequently encountered in a clinical setting. These organisms cause both community and hospital acquired infection. In the past 2 decades, the global emergence and spread of antimicrobial drug resistance among Enterobacteriaceae was mostly associated with extended-spectrum β-lactamases (ESBLs) and carbapenemases (Lynch et al., 2013). New Delhi Metallo-β-lactamase-1 (NDM-1) is a gene encoding for a carbapenem hydrolysing

enzyme which was first described in 2009 in *Klebsiella* and *E. coli* isolates from a Swedish patient who had travelled to and was hospitalised in New Delhi, India (Yong et al., 2009). The first case with NDM-1-producing Enterobacteriaceae was reported in Vietnam in September 2010 (Hoang et al., 2013).

During April – July 2010, data from 4 countries in SEA (Philippines, Singapore, Thailand and Vietnam) in hospitalised patients showed that the proportion of ESBL positive Enterobacteriaceae ranged from 19.8% (Singapore, 96 isolates) to 55.1% (Vietnam, 71 isolates) whilst the proportion of carbapenem resistant Enterobacteriaceae ranged from 2.9% (Philippines, 70 isolates) to 5.6% (in Vietnam) (Suwantarat and Carroll, 2016).

In a national antimicrobial resistance surveillance program between 2011 and 2015, the proportion of carbapenem resistant *K. pneumoniae* increased from 2% to 12% in Singapore, from 6% to 11.9% in Philippines, from 0.5% to 1.6% in Malaysia and 0.3% to 4.9% in Thailand (Hsu et al., 2017). In the same period, the proportion of carbapenem resistant *E. coli* rose from 0% to 5% in Singapore whilst it remained unchanged in Philippines (6%) and in Thailand (0.8% to 0.9%) and decreased from 0.5% to 0.2% in Malaysia (Hsu et al., 2017). Data on CRE in other countries in SEA (Brunei, Cambodia, Timor-Leste and Laos) was not available (Hsu et al., 2017).

1.2. The growing crisis of hospital acquired infections

1.2.1. Overview of hospital acquired infections (HAIs)

A hospital acquired infection or nosocomial infection is defined as one acquired in hospital by a patient who was admitted for other reasons and that infection is not presented or not in incubation on admission (Ducel et al., 2002). A cut-off point of 48 hours after admission is widely used to distinguish between community and hospital acquired infections. In addition, the term healthcare-associated infection (HCAI) was originally proposed to refer to infections, which occur in patients on admission or within 48 hours of admission having had previous exposure to invasive procedures or healthcare facilities (such as nursing homes, outpatient treatment facilities etc) (Friedman et al., 2002). Currently, such facilities are uncommon in Vietnam and many LMICs and thus this thesis will only focus on HAIs.

HAIs are classified into anatomical site-specific infections and device related infections: surgical site infections, urinary tract infections (UTIs and UTIs related to urinary catheters) respiratory infections (hospital acquired pneumonia (HAP) and respiratory infection related to an endotracheal or tracheostomy tube), vascular catheter site infections (both peripheral and central) and blood stream infections (BSIs and those related to vascular catheters) (Centers for Disease Control and Prevention (CDC), 2013b, Ducel et al., 2002). The US Centers for Disease Control and National Healthcare Safety Network (CDC/NHSN) has grouped HAIs into 14 major categories with 55 infection types (Centers for Disease Control and Prevention (CDC), 2013b) whilst the European Centre of Disease Prevention and Control (ECDC) defined 56 types of HAIs in ICUs. At a minimum, reporting is required for bloodstream infection and pneumonia, urinary tract infections and catheter-related infections may be added optionally (European surveillance of healthcare-associated infections in intensive care units, 2015). Nomenclature and definitions vary between these two organisations and Vietnam has utilised both.

Hospital acquired infections affect all health care facilities in the world and have become a major cause of mortality and morbidity among inpatients and represent an important patient safety issue (Allegranzi et al., 2011). HAIs impact on length of stay, cost of treatment and outcome. However, the management of HAI is complex, in terms of clinical diagnosis, microbiological diagnostics, the growing proportion of antimicrobial resistant pathogens and the selection of appropriate empiric antimicrobials, particularly in patients requiring mechanical ventilation (Harmanci et al., 2002). The accurate aetiological diagnosis of HAIs, particularly in LMICs, is challenging because of the need for high quality microbiological investigations on specimens from all possible sites of infection.

Hospital acquired respiratory infections are sub-classified depending on whether they are related to mechanical ventilation and intubation. Ventilator associated pneumonia (VAP) is a term used for pneumonia that occurs at least 48 hours after endotracheal intubation; ventilator associated tracheobronchitis (VAT) is a term used for other lower respiratory tract infections, such as tracheobronchitis or tracheitis without evidence of pneumonia (Centers for Disease Control and Prevention (CDC), 2013b).

VAP is a particular problem in ICUs throughout the world. The development of VAP involves multiple factors and a complex interaction between the endotracheal tube, patient's immunity and pathogen invasion into the lower respiratory tract. Pathogens can

spread to the lower respiratory mucosa causing infection via different mechanisms through (1) aspiration of infected respiratory secretions or gastric contents, (2) direct extension of a nearby infection, (3) inhalation of contaminated air or (4) by hematogenous dissemination from other remote local infection (Safdar et al., 2005a). Pathogenic bacteria may be acquired through endogenous routes (caused by host pathogenic flora) or exogenous routes (acquisition of bacteria from health care activities, invasive interventions or hospital environment). Based on understanding of these mechanisms, several strategies for preventing VAP have been developed as discussed later in this thesis.

Differentiation of colonization and infection is important in diagnosis of HAI and making decisions on antibiotic therapy but is challenging in clinical practice (Figure 1-3). For example, Gram-positive bacteria are often isolated from the upper respiratory tract whilst the lower respiratory tract is considered sterile and therefore any significant isolates from the lower respiratory tract will be treated as pathogens (Robinson, 2004). However, the challenge is that specimens from the trachea and lung are often contaminated with organisms from the upper respiratory tract, or that have colonised the endotracheal tube, as the specimen is obtained. Both invasive and non-invasive diagnostic methods are widely used for diagnosis of VAP. As recommend by the American Thoracic Society since the first consensus statement on management of HAP in adults, blood cultures were used in an investigation of aetiological agents but positivity of bloodstream infection in an episode of VARI varied from 8-20% (American Thoracic Society, 1996). The microbiological diagnosis of VARI can be made by non-invasive methods, such as endotracheal aspiration (ETA) or invasive methods, such as protected specimen brush (PSB) or bronchoalveolar lavage (BAL) (Kalil et al., 2016). The clinical judgement of colonization or infection, backed by microbiological methods such as the identification of inflammatory cells in samples and the absence of epithelial cells (associated with the mouth and oropharynx) and use of (semi-) quantitative culture play important roles in the decision to start antibiotic treatment (Nseir et al., 2010). Additionally, distinguishing new clinical infection from colonization is required to measure the incidence of HAI which is the gold standard for HAI surveillance in past decades, although it is time consuming and expensive (Zingg et al., 2014). Thus, collection of samples and bacteria alone is insufficient for the accurate recording of HAI rates, particularly in the respiratory tract. Quantitative culture of respiratory samples is often used to differentiate between infection and colonization with widely accepted thresholds for the diagnosis of pneumonia as follows: ETA samples $\geq 10^5$–10^6 colony forming units

(CFU)/ml; PSB samples ≥10^3 CFU/ml and BAL samples ≥10^4 CFU/ml (Ioanas et al., 2001). A Cochrane systematic review of five RCTs comparing respiratory samples processed quantitatively or qualitatively with 1367 patients showed no clinical advantage (in terms of reduction in mortality, ICU length of stay and duration of mechanical ventilation) of invasive diagnostics or quantitative cultures of respiratory secretions over non-invasive diagnostics or qualitative cultures in patients with suspected VAP (Berton et al., 2012). The complex diagnostic definitions for VAP which often require radiological, clinical and microbiological evidence make both high-quality epidemiology and research challenging and lead to bias due to the subjectivity in these definitions.

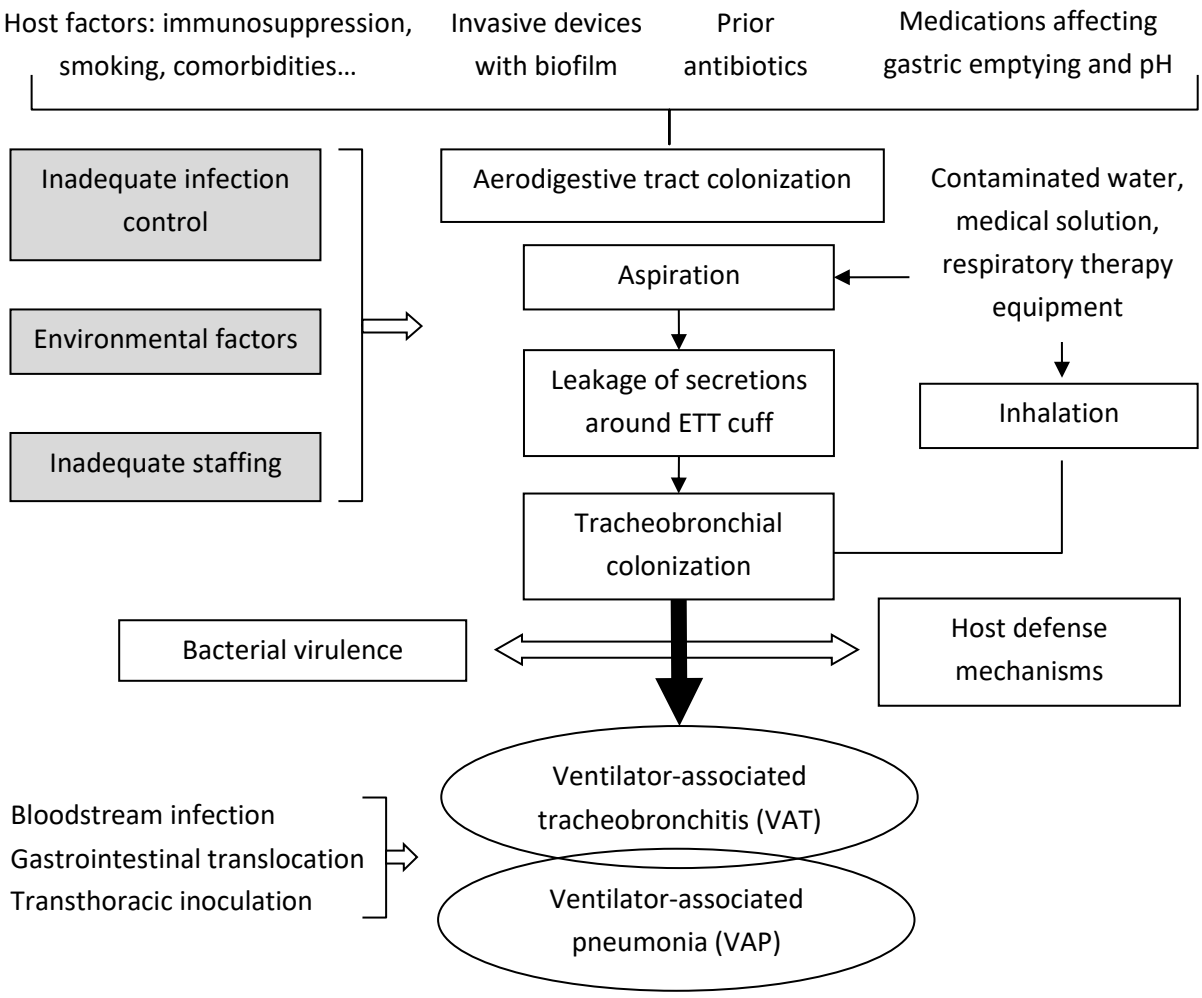


Figure 1-3. Pathogenesis of ventilator-associated respiratory infection (VARI).

Adapted from Craven and Kollef (Craven et al., 2009, Craven and Steger, 1995, Kollef, 1999).

1.2.2. Epidemiology of hospital acquired infection (HAI)

Limitations in design and the low quality of data may result in poor estimates of HAI burden in LMICs. In a systematic review of health-care-associated infection in LMICs, 118/220 (54%) of reviewed articles from 1995 to 2008 in Medline were graded as low quality (Allegranzi et al., 2011). The pooled prevalence of HAIs was double to triple that in high-income settings as Europe and the US (15.5 vs 7.1 and 4.5 per 100 patients, respectively). In this review, *Pseudomonas* (29%), *Enterobacteriaceae* (21%) and *Acinetobacter* species (24%) were leading causes of ventilator associated pneumonia (VAP) whilst bloodstream infections (BSI) were caused largely by *Staphylococcus aureus* (19%), *Acinetobacter* species (18%), Coagulase-negative *staphylococci* (17%) and *Enterobacteriaceae* (18%) (Allegranzi et al., 2011). Recent data from 43 countries with different income levels, including 503 ICUs in 2007-2012 showed high overall HAI prevalence (the overall rate of VAP per 1,000 mechanical ventilator days was 14.7 (95% CI, 14.5-14.9)) (Rosenthal et al., 2014).

We performed a literature search of 2 database (Embase and Ovid) from January 1990 to August 2017 for VAP in Asia for studies with period prevalence data, incidence density data, microbiology data and any data on costing of VAP (Bonell et al., 2018). Asian countries were defined according to the World Bank classification of income level at the time the study was published and further defined as tropical or non-tropical based on their geographical latitude. Period prevalence was highest in upper middle income countries (UMIs) and lowest in high income countries (HICs) (prevalence rate by country and region was shown in Figure 1-4). In this systematic review using data from 14 countries in Asia from 1990 to 2017, the point incidence density of VAP was 15.1 episodes per 1000 ventilator-days (95%CI 12.1–18.0) (Bonell et al., 2018). The incidence density of VAP was high in both LMICs and UMICs, and there was no indication of a reduction over time on meta-analysis. However, the incidence density of VARI in LMICs was higher than in HICs (18.5 vs 9.0 per 1000 ventilator-days; P = 0.035) (Bonell et al., 2018).

From this review, we concluded that VAP is a major contributor to HAIs in Asia. Among 14,295 organisms were identified, *A. baumannii* was the most common (26%), followed by *P. aeruginosa* (22%], *K. pneumoniae* (14%), and *S. aureus* (14%). In LMICs, *A. baumannii* was the most common. In tropical regions, *A. baumannii* was the most common species whilst *S. aureus* was a more common in - regions. Given the large expansion of ICUs in these

settings, the at-risk population can be expected to rise substantially in the immediate future, compounding the problem.

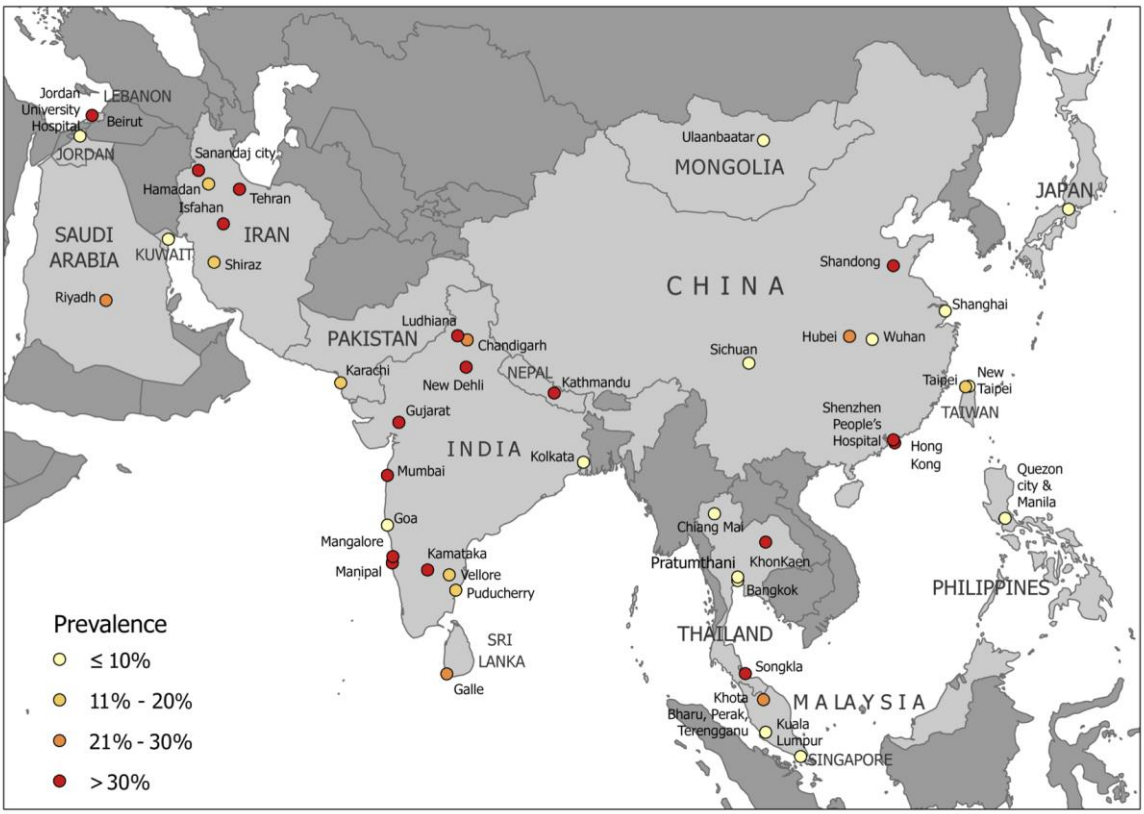


Figure 1-4. The prevalence rates of ventilator-associated pneumonia in Asia.

Southeast Asia is a region with high population density. The burden of HAIs in this region is not well documented, with even less literature concerning the lower income countries within this region (Ling et al., 2015). The overall prevalence of HAIs in Southeast Asia was reported as 9% in a recent meta-analysis, which is lower than in high income countries (15.5%), potentially due to systematic errors in reporting (Ling et al., 2015).

In Thailand, the average point prevalence of HAIs was 6.5%, with the highest prevalence observed in ICU (22.6%) (Danchaivijitr et al., 2007). The most common HAIs were hospital acquired pneumonia/VAP, urinary tract infection and bloodstream infection. VAP was the most common device-associated infection with incidence densities of 10.8-13.6/1,000 ventilator days (Reechaipichitkul et al., 2013, Danchaivijitr et al., 2007). Pathogens were isolated from around 70% of all HAIs and there was a predominance of Gram-negative bacteria (70% of isolates) (Reechaipichitkul et al., 2013). The types of HAI and detected bacterial isolates were similar in different studies. The most commonly isolated bacteria from patients with HAP and VAP were *P. aeruginosa* and *A. baumannii* (Reechaipichitkul et

al., 2013, Werarak et al., 2010). HAP and VAP were associated with a 30-day mortality of 45.9% compared to 2.7% in those without infection (Werarak et al., 2010). Hospital acquired blood stream infection (HABSI) also were associated with higher ICU mortality in Thailand where overall mortality was 28.3%. The leading HABSI pathogens were *E. coli* (17.4%), *S. aureus* (15.2%), *K. pneumoniae* (12.3%) and *P. aeruginosa* (10.3%). Although the prevalence of HABSI decreased from 15.5/1000 (in 1987) to 12.2/1000 hospitalizations (in 2003), the mortality remained unchanged, perhaps partly due to the increasing occurrence of multidrug resistant pathogens (Hortiwakul et al., 2012). Results from the point-prevalence studies in Southeast Asia are presented in Table 1-5.

Table 1-5. Overall type of hospital acquired infections from point-prevalence studies in selected countries in Southeast Asia

HAIs	**Thailand (Danchaivijitr et al., 2007)**	**Malaysia (Hughes et al., 2005)**	**Vietnam (Phu et al., 2016)**	**Singapore (Cai et al., 2017)**
Study setting	20 hospitals in 2006	Single site in 2001	15 adult ICUs in 2012-2013	13 hospitals in 2015-2016
Study population (patients)	9865	747	3287	5415
Type of HAIs				
Hospital acquired pneumonia (HAP)	36.1%	21.4%	79.4%	24.8%
Urinary tract infection (UTI)	25.5%	12.2%	2.9%	6.7%
Bloodstream infection (BSI)	9%	12.2%	4.4%	8.7%
Surgical site infection	11.0%	11.2%	4.2%	17.3%
Skin and soft tissue infection	7.7%	4.0%	1.5%	7.2%
Pathogens identified	70.8%	68%	59.6%	45.5%
Etiology of HAIs				
P. aeruginosa	13.4%	21.4%	13.8%	11.5%
Acinetobacter species	10.7%	3.43	24.4%	6.7%
MRSA	5%	9.1%	5.4%	7.5%
K. pneumoniae	10.9%	5.1%	11.6%	10.2%
E. coli	6.9%	4.6%	5.4%	10.4%

MRSA: Methicillin-resistant *Staphylococcus aureus*.

1.2.3. Economic burden of hospital acquired infections

The excess cost associated with HAIs varied among different countries. A systematic review of the economic impact of HAIs on the US health care system in 2012 showed that the annual hospital costs of 5 major HAIs (surgical site infections, central line-associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonia and *C. difficile* infections) was US$ 9.7 billion. In which the estimated cost attributable to VAP was US$ 40,144 per case (95% CI 36,286–44,220), with a total economic burden of US$ 3.1 billion (95% CI US$ 2.8-3.4 billion) (Zimlichman et al., 2013). Meanwhile, the additional amount of money spent for an episode of VAP in Europe is estimated to be around US$ 13,000–15,000 (Arefian et al., 2016a, Wyncoll and Camporota, 2012). For Asian countries, data on the cost of VAP management was limited. In our recent systematic review, we found only 5 studies reporting the economic burden of VAP with different methodologies (Bonell et al., 2018). Three studies compared the average cost between patients with and without VAP, respectively which was US$6,251 vs. US$2,599 in India (Mathai et al., 2015), US$8,925 vs. US$6,626 in Taiwan (Tzeng et al., 2015) and 3.04 purchasing power parity (PPP, a measure of the total amount of goods and services that a single unit of a country's currency can buy in another country) per day vs. 1.23 PPP per day in Iran (Karkhane et al., 2016). One study in India calculated that the average excess cost of antibiotics used to treat VAP was US$433 (Misal et al., 2017) and one study in Thailand reported the average hospital cost per patient admitted to ICU before and after implementation of the care bundle for VAP $4,769 and $2,378 respectively (Apisarnthanarak et al., 2007). In Vietnam, the excess cost of HAI ranged from US$ 865 to US$ 1,131 in the period of 2008 to 2010 (Ha and Ha, 2012, Thi Anh Thu et al., 2015) and in a study of 3 Vietnamese tertiary referral hospitals from 2013 to 2015, the cost difference between patients with and without VAP was estimated at US$ 2,189 (Phu et al., 2017).

Given the differences in methodologies and in country incomes among these studies, the economic impact on the healthcare systems can only be compared in a limited way. The variations in excess cost may be due to differences in costing of used resources and the healthcare systems. Estimating the excess cost associated with HAIs could help inform policy makers in planning and implementing necessary changes in infection prevention and control, antibiotic stewardship programmes, resource allocation and other relevant policies with the final purpose to reduce the incidence and burden of HAIs (Graves, 2004, Arefian et al., 2016b). A number of studies have been conducted across the world to answer

the question regarding the excess financial cost of treating HAIs, with certain variations in methods or definitions. The approaches to estimate the economic burden of HAIs have 2 major limitations: lack of a distinction between cost attributable to the management of HAIs and cost attributable to the management of the causes of initial hospital admission and lack of an adequate method for calculating indirect costs (e.g. lost wages, incapacitation, lost future wages or premature death) (Gianino et al., 2007, Marchetti and Rossiter, 2013).

Costs attributable to HAIs can be estimated using the concurrent method (examining individual patients and itemising all resources used during an HAI episode) or comparative method (comparing the cost of patients with HAIs and patients without HAIs or "controls") (Haley, 1991). The first method is time consuming and requires detailed protocols to determine the events which are attributable to HAIs (Haley, 1991, Graves et al., 2010). The latter method is less time-consuming but has 2 difficulties: 1) several factors (e.g. comorbidity) can prolong the hospital stay regardless of HAI but these factors are hard to match and 2) for matching these factors, it increases number of matching factors and therefore can exclude the patients with HAI because the controls is exhausted (Graves et al., 2010).

These costs include the hospital costs, hospital charges, resources used and actual re-imbursement, and the hospital cost is a useful indicator as they reflect the actual economic burden of HAI (R. Douglas Scott II, 2009). For each episode of HAI, this cost should take into account the length of stay associated with HAIs, type and dosage of medication administered to treat the infections and number and type of services for diagnostics of the HAI episode (Gianino et al., 2007).

1.2.4. Diagnostics of hospital acquired infections

Microbiological diagnostics play an important role in the control of HAIs in 3 ways: screening, detection and surveillance. In screening, diagnostics help to identify patients with colonisation of resistant pathogens and these patients can be placed under stricter infection prevention and control measures, such as standard precautions and isolation to prevent the spread of resistant organisms (Siegel et al., 2007). In detection, rapid high quality laboratory diagnostics play an important role in the management of infection by

enabling accurate diagnosis of aetiology and antimicrobial susceptibility, guiding appropriate treatments for individual patients and informing locally relevant guidelines, (Petti et al., 2006, Caliendo et al., 2013). Diagnostics are also important in facilitating stewardship and enabling surveillance of antimicrobial resistance (AMR) (Tsalik et al., 2016).

LMICs, where the burden of HAIs and AMR is highest, often lack laboratory capacity, both in terms of equipment and highly trained laboratory staff, required to provide accurate microbiological diagnoses, resulting in lack of trust in results and underuse of microbiological diagnostics (Petti et al., 2006, Peterson et al., 2001). This leads to limited knowledge of the causes of HAIs, of the spread and significance of antimicrobial resistance and may lead to inappropriate use of antibiotics with impacts on both AMR and mortality and morbidity. Advanced diagnostics are needed for addressing the antimicrobial resistance threats and reducing operational requirements in LMICs (Ming et al., 2019).

The rapid detection and identification of bloodstream pathogens is important to establish the diagnosis of bacteremia and guide treatment. However, blood culture detects pathogens in < 50% of patients with sepsis (Kayange et al., 2010, Heffner et al., 2010). Increased time to notification of positive blood culture is associated with increased length of hospitalization (Beekmann et al., 2003). The early, accurate identification of bloodstream infection assists in rapid deployment of optimal antibiotic therapy for these patients with life threatening infections.

Microbiological molecular methods have potential advantages over conventional techniques, in terms of speed, throughput, sensitivity, quantitation and automation (Ecker et al., 2010). Current available new techniques for identification of pathogen in blood are often based on the detection of proteins (such as Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) or nucleic acids (such as fluorescence in situ hybridization (FISH), microarray and real-time PCR) (Loonen et al., 2014).

MALDITOF-MS has been recognized as a revolution in microbiological diagnostics since it was first developed by Nobel laureate Koichi Tanaka in the 1980s (Tanaka et al., 1988). In the hospital setting, the rapid availability of accurate identification and susceptibility testing of bacterial (and fungal) pathogens is important to guide appropriate therapy. A diagnostic system that is robust, accurate, rapid and has low running costs, could be cost

saving and improve patient outcomes. MALDITOF uses a proteomic method to identify pathogens by analyzing microbial colonies which grow from culture media, blood culture broth, cerebrospinal fluid (CSF) or urine (Clark et al., 2013). The sample is prepared by addition to a matrix of a laser energy-absorbent, low mass organic compound in the MALDITOF metal target plate and air dried at room temperature for co-crystallization before insertion into a mass spectrometer. The sample-matrix crystal is bombarded by an ultraviolet laser causing sublimation and ionization. The mass analyzer detects characteristics of sample protein ions through their movement in the time of flight (TOF) tube to produce a spectrum of mass-to-charge (m/z) ratios. The pathogen protein profile is as distinctive as a fingerprint and is matched to either identical or the closest spectra in a reference database to identify genera, species and even strain types (Clark et al., 2013, Bizzini and Greub, 2010, Singhal et al., 2015). In addition to replacing the need for expert microbiologists and expensive reagents, MALDITOF also offers the valuable advantage of reducing the time to identification of pathogens (TTD) over conventional biochemical methods of identification in LMICs (see Supplementary Table 2). It allows to identify pathogens from positive blood cultures within 20 minutes.

1.3. Interventions to reduce AMR and HAI in hospital

1.3.1. Antimicrobial stewardship programmes (ASP)

Antimicrobial stewardship is described as a healthcare-system-wide approach to judicious use of antimicrobials to preserve their future effectiveness (National Institute for Health and Care Excellence (NICE), 2015). It includes coordinated interventions designed to improve and measure the appropriate use of antimicrobials by optimising its dose, duration of therapy, route of administration and maximising clinical outcomes whilst minimising toxicity and selection of resistant organisms (Barlam et al., 2016, World Health Organization. Regional Office for South East Asia, 2011).

There is a correlation between antimicrobial resistance and antimicrobial consumption (Davies and Davies, 2010). Antibiotic prescription data from outpatients in 18 European countries between 1997 to 2002 showed a significant correlation between prevalence of penicillin-resistant *S. pneumoniae* and consumption of penicillin, with higher resistance rates in higher consuming countries (Goossens et al., 2005). A similar trend between

pneumococcal resistance rates and consumption of beta-lactam and macrolide antibiotics was reported between 1998 and 1999 in another study utilising 482 laboratories from 23 countries in the European Antimicrobial Resistance Surveillance System (EARSS) and national outpatient sales data for antibiotics gathered from 13 member states of the European Union (Bronzwaer et al., 2002).

The hospital setting is an important one for AMR as it is the setting where antibiotic use and resistance rates are highest. As a result of antibiotic use prior to and on admission to hospitals, infections acquired within the hospital setting (hospital acquired infection) tend to be associated with bacteria that are resistant to more antibiotics than those acquired in the community. The likelihood is that if 'untreatable' bacteria are to arise, it will be in the hospital setting, more than likely within the intensive care department where broad-spectrum antibiotic use is highest and patients are extremely vulnerable. A recent study looking at Dutch travellers who acquired faecal carriage of resistant bacteria whilst abroad found a 12% risk of spread of ESBL producing *Enterobacteriaceae* from the traveller to household members on return (Arcilla et al., 2016) If similar levels of transmission in the household are seen in hospital discharges from LMICs, and carriage is sustained, then rapid spread of resistance to the community should be anticipated, further emphasising the importance of controlling AMR in the hospital setting.

In hospital settings, broad-spectrum antibiotic consumption was also reported as associated with high resistance rates among the Gram-negative bacilli and Gram-positive cocci (Loeffler et al., 2003, Hsu et al., 2010). The exposure to many antibiotics in the hospital environment results in selective pressure, encouraging the emergence, persistence and transmission of multidrug resistant pathogens. Antibiotic restriction and cycling strategies have been implemented and may contribute to decreasing the total number of antibiotic-resistant pathogens causing HAI (Gruson et al., 2000, Lai et al., 2015) or bringing resistance rates back to baseline levels, although their efficacy in improving clinical outcome has been inconclusive (Brown and Nathwani, 2005).

In 2016, the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) recommended 5 fundamentals of antimicrobial stewardship (Barlam et al., 2016). Firstly, administrative interventions include the use of audit and feedback, development and implementation of facility specific guidelines targeting common infections, education, routine prescriber-led review of

antibiotic prescribing and use computerized clinical decision support systems. Secondly, optimisation of antibiotics use is recommended by implementing PK monitoring and adjustment programs for aminoglycosides and vancomycin and using the shortest effective antibiotic therapy duration. Thirdly, the microbiology and laboratory diagnostics is recommended by developing stratified antibiograms and selective and cascaded reporting of antibiotics, using rapid diagnostics testing in additional to conventional cultures and serial procalcitonin measurements. Fourthly, measurement of interventions should include monitoring antibiotic use and its cost and measurement of interventions on clinical outcomes. Finally, antimicrobial stewardship needs to be considered in some special populations of neonates, immunocompromised patients and hematology-oncology patients.

1.3.2. Infection prevention and control interventions

HAI prevention strategies often focus on implementing standard precautions among health care staff to limit transmission of pathogens, controlling the risk of infection from the hospital environment and invasive medical interventions, and promoting optimal antibiotic therapy in hospital and training staff (Ducel et al., 2002).

In ICUs, the most common HAI are ventilator associated respiratory infections (VARIs) (Ling et al., 2015, Allegranzi et al., 2011). A substantial reduction in this would provide direct benefit to patients and potentially reduce the exposure to very broad-spectrum antibiotics, limiting the generation of resistance to them and potentially delaying the emergence of completely resistant bacteria. In high income settings 'care bundles' have been developed to deliver improvements in many aspects of critical care, including prevention of VAP. Care bundles for VAP prevention may include essential components of surveillance, diagnosis, treatment and prevention for VAP, based on available evidence and expert opinion, and combined in an effort to improve compliance in implementation. In general, implementation of VAP care bundles has been shown to be feasible, with compliance increasing over time since their first introduction and now achieved in more than 70% of units in some high income countries (Sen et al., 2016, Morris et al., 2011, Bird et al., 2010, Alsadat et al., 2012). Implementation of VAP care bundles was associated with a significant reduction in VAP rate (reduced from 10.2 cases/1000 ventilator days to 3.4 cases/1000 ventilator day) (Bird et al., 2010) and in antibiotic use (Morris et al., 2011), saved cost (Bird et al., 2010) and contributed to a decrease in the incidence of MRSA colonization (from

10% to 3.6%) (Sulis et al., 2014, Morris et al., 2011) (see further discussion on care bundles in Chapter 7, 7.1.Introduction). However, there is little data on the effectiveness of even the components of such bundles in the SE Asian resource constrained setting, where staffing levels are often substantially different, the opportunity for isolation lacking, background levels of antimicrobial resistance higher, and some interventions proven to work in developed world settings, such as semi-recumbent patient positioning, have failed to demonstrate effectiveness when scrutinized in these settings (Loan et al., 2012).

In 2014, the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) released recommendations on VAP prevention, updating guidance from 2008 (Klompas et al., 2014) with the most recent update in 2016 (Kalil et al., 2016). Recommendations of VAP prevention was presented in Table 1-6. Among 16 interventions recommended to prevent VAP and other ventilator-associated events, 5/16 recommendations were graded “low quality of evidence”. These will require more attention from researchers to develop sufficient evidence to improve further strategies to prevent VAP.

Maintenance of endotracheal cuff pressure through continuous control of endotracheal cuff pressure is listed as an approach to reduce VAP through prevention of the aspiration of contaminated oropharyngeal secretions. This would be an ideal strategy for a resource constrained setting as the technique involves a reduction in the nursing input (as compared with subglottic suction which requires an increase in nursing interventions) and has little cost in terms of disposable items (as compared to innovative endotracheal tubes, which carry a high disposable cost and would require a change in culture to minimize wastage). However, previous studies (see Table 1-6) have not established sufficient evidence to recommend this intervention. Of note, many strategies, including continuous control of endotracheal cuff pressure, have not been shown conclusively to impact on antibiotic use, a crucial feature when trials are unblinded, and ‘VAP prevention’ may simply be the result of re-categorisation of HAI into another, non-respiratory, diagnosis (Nseir et al., 2011, Lorente et al., 2014).

Table 1-6. Summary of recommended strategies for preventing Ventilator-Associated Pneumonia (VAP) (Klompas et al., 2014, Alvarez Lerma et al., 2014, Coffin et al., 2008)

Intervention	Impact on VAP rate	Quality of evidence	Ref
General strategy:			
Education and training	Reduced VAP rate by 57.6%-59%	Moderate	(Zack et al., 2002, Apisarnthanarak et al., 2007)
Hand hygiene	Reduced VAP rate 50.6% (29.8%-65.5%)	Moderate	(Ma et al., 2014)
Use of non-invasive positive pressure ventilation in selected populations	Reduced VAP rate 38% (20%-73%)	High	(Hess, 2005)
Facilitate early mobility	Reduced VAP rate from 2.14 $\pm$ 0.95/1000 ventilator-days to none	Moderate	(Titsworth et al., 2012)
Bed head elevation	A benefit favored to the 45° bed head elevation (RR = 0.47, 95% CI = 0.19-1.17) in reducing VAP rate	Low	(Niel-Weise et al., 2011)
Prophylactic probiotics	Reduced VAP rate (RR 0.75; 95%CI 0.59–0.97)	Moderate	(Petrof et al., 2012)
Strategies to prevent micro-aspiration			
Utilize endotracheal tubes with subglottic suctioning	Reduced VAP rates by 55% (46-66%)	Moderate	(Muscedere et al., 2011)
Automated control of endotracheal tube cuff pressure	Reduced VAP rate from 26.2% to 9.8%	Low	(Nseir et al., 2011)
Maintain an endotracheal cuff pressure of at least 20 cm H2O	Persistent intracuff pressure below 20 cm H20 increased VAP (RR=4.23, 95% CI 1.12-15.92)	Low	(Rello et al., 1996)
Ultrathin polyurethane endotracheal tube	Reduced VAP rate by 50%	Low	(Poelaert et al., 2008)
Strategies to reduce colonization of the aerodigestive tract			

Intervention	Impact on VAP rate	Quality of evidence	Ref
Regular oral care with chlorhexidine	Reduced VAP rate by 60% (47%-77%)	Moderate	(Shi et al., 2013)
Strategies to minimize contamination of equipment			
Change the ventilator circuit only if visibly soiled or malfunctioning	No change in VAP rate	High	(Kollef et al., 1995)

Whilst attention has focused on VAP, it is important to understand that in many settings in SEA ventilator associated tracheobronchitis (VAT) is also treated with antibiotic therapy, with the aim of preventing the development of VAP and prolonged ventilation time. Whilst this may be considered as contentious by some, there is some evidence behind this approach (Nseir et al., 2011). Interventions that prevent VAP should also prevent VAT, due to their shared aetiological pathway, and thus the target for such interventions can reasonably be considered as Ventilator Associated Respiratory Infections (a combination of VAP and VAT) (Martin-Loeches et al., 2015, Craven and Hjalmarson, 2010).

A randomized controlled trial will provide an opportunity to robustly evaluate the impact of continuous pressure control, not just in preventing ventilator associated respiratory infections but also in reducing antibiotic use.

1.4. Situation of hospital acquired infections, antimicrobial use and resistance in Vietnam

Vietnam is ranked by the World Bank as a lower middle-income country and, with a population of 91 million people, it is the 13th most populous country in the world and the third-largest population in Southeast Asia (World Bank, 2016). The number of annual hospital admissions ranges from 6,000-14,000 per hospital of which critical care admissions usually account for 5-18% of all admissions. The number of critical care unit (CCU) beds accounted for 4-11% of total hospital beds whilst the number of doctors and nurses per actual CCU bed was 0.22 and 0.56, respectively (Dat et al., 2017a). Along with the lack of human resources, hospital overcrowding and inappropriate antimicrobial use, there are also gaps in the available infection control policies, low compliance with hand hygiene

among healthcare workers and a lack of surveillance systems together contributing to the spread of antibiotic resistance and hospital acquired infection in Vietnamese hospitals (Johansson et al., 2011, The GARP Vietnam National Working Group, 2010).

Several studies have assessed antibiotic use and resistance and HAI incidence in hospitals and in ICUs and shown increasing trends. I have summarised these studies below.

A point prevalence study among 36 hospitals in Vietnam (2 national-level, 18 provincial-level and 16 district hospitals) showed that 64.7% of hospitalised patients were receiving antibiotics and 30.8% of these were considered inappropriate (Thu et al., 2012). The antimicrobial use was even higher in ICU patients (85%) in our multi-ICU study (Phu et al., 2016).

In 2007, in a one-day cross sectional study among 7,571 patients in 36 hospitals in Vietnam, the HAI prevalence was 7.3% (553/7571) and HAP was the most common HAI (41.9%) followed by surgical wound infections (27.5%). Oral endotracheal intubation and tracheotomy were independent risk factors for respiratory infections (adjusted OR=1.6 and 10.9, p values < 0.01 respectively) (Truong Anh Thu et al., 2009b). Data from a single ICU in a tertiary, referral 1400-bed hospital during this period showed 21.2% (172/808) patients had HAP and 492/808 patients (61%) were on ventilation. Of the 172 patients with HAP, 144 (84%) were VAP. The most commonly detected isolates were *A. baumannii* (41.5%), *P. aeruginosa* (27.9%), and *K. pneumoniae* (9.5%). HAI was associated with increased hospital length of stay (26.6 days vs 11.5 days) and extra hospitalization costs (2,385 vs 1,114 US dollars) (Truong Anh Thu et al., 2009a).

During an active surveillance study in seven large hospitals in Vietnam from 2008-2010, surgical site infection was observed in 5.5% of surgical procedures, with the highest rates in colon surgery (33.3%), limb amputation (25%) and vascular surgery (25%). Among pathogens isolated from surgical site infections, the most frequent isolates were *E. coli* (39%) with a carbapenem resistance rate of 20%, followed by *Enterococcus* spp. (10%) and *A. baumannii* (8%) (Viet Hung et al., 2016).

From 2012-2013, HAI prevalence was reported as 29.5% in our multi-ICU study with the most common infection being HAP (79.4%), followed by blood stream infection (4.4%) and surgical site infections (4.2%). Gram-negative bacteria were responsible for 84.2% of all isolates. *A. baumannii* and *P. aeruginosa* were the most common pathogens causing

pneumonia (25% for *A. baumannii* and 15.7 % for *K. pneumoniae*) whilst *A. baumannii and E. coli* were frequently isolated in BSI (22.7% and 13.6% respectively) (Phu et al., 2016).

Beside large multi-centre studies mentioned above, ICU based studies, mostly conducted in the National Hospital for Tropical Diseases in Hanoi and the Hospital for Tropical Diseases in Ho Chi Minh City reported high prevalence and high resistance rates of *A. baumannii*, *P. aeruginosa* and *Enterobacteriaceae* as a most common isolates from ETA and bloods in critically ill patients over past 10 years (see Table 1-4).

In the Vietnamese hospital setting, the most highly resistant bacteria were *Acinetobacter*, *Klebsiella* species, *E. coli* and *Pseudomonas* species (Phu et al., 2016, Le et al., 2016, Johansson et al., 2011). The proportion of *A. baumannii* with carbapenem resistance ranged from 51-93.2% in ICU studies (Table 1-4). Two studies reported colistin resistance among *A. baumannii* at 11.2% (13/116) and 6% (6/101) (Tada et al., 2013, Tuan Anh et al., 2017) and notably, one hospital reported a high prevalence of colistin resistance at 31.6 % (12/38) (Tuan Anh et al., 2017). The proportion of *P. aeruginosa* isolates showing carbapenem resistance was from 42.9 to 86.2% (Table 1-4).

A multi- centre paediatric ICU study reported high prevalence of carbapenem resistance in HAIs caused by *P. aeruginosa* (69% or 25/29), *A. baumannii* (67% or 24/36) and *K. pneumoniae* (55% or 23/42). Among 95 *Enterobacteriaceae* isolates, 88% were resistant to 3rd-generation cephalosporins and 40% were resistance to both cephalosporins and carbapenems. The prevalence of methicillin-resistance among Staphylococcus aureus (MRSA) was 81% (13/16) (Le et al., 2016).

In an 11 year retrospective study (2000 – 2010) in an infectious disease ICU in southern Vietnam, *Acinetobacter* was recognised as the most common pathogen (30.4%, n=206) isolated from tracheal aspirate samples in suspected VAP patients with an average annual increase of 6.6% (Nhu et al., 2014). From 2008, the proportional of *Acinetobacter* spp. isolates dramatically increased by 66% and carbapenem resistance was almost 80%.

In a study in northern Vietnam at 3 ICUs with 62 beds from 2007-2008, *Acinetobacter* was identified in 112 (29.4%) respiratory tract isolates and 21 (14.4%) blood isolates, of which *A. baumannii* accounted for 88/112 (78.6%) and 16/21 (76.2%) isolates respectively. The resistance rate among *Acinetobacter* isolates to ceftazidime, ciprofloxacin, gentamicin and imipenem was 89%, 80.1%, 79.1% and 69.1% respectively (Johansson et al., 2011).

From October 2012 to September 2013, our monthly repeated point prevalence survey of 15 adult ICUs in Vietnam also showed the most frequent isolates in HAIs was *A. baumannii* (24.4%, 177/726) with the carbapenem resistant prevalence of 89.2% (149/167) (Phu et al., 2016).

During a study period between 2012 and 2014, 119/160 (74.4%) *A. baumannii* isolates from 3 hospitals in southern Vietnam were classified as MDR (resistant to at least 1 agent in 3 or more antimicrobial families), of which 117/119 (98.3%) included imipenem resistance. Colistin resistance was exhibited in 13/116 (11.2%) isolates (Tuan Anh et al., 2017).

Vietnam reported the first cases of the New Delhi metallo-beta-lactamase 1 gene (NDM-1) producing *E. coli* and *K. pneumoniae* from 2 patients who admitted to a surgical hospital in September 2010 (Hoang et al., 2013). In a study of 2233 patients admitted to 12 Vietnamese hospitals during 2017 and 2018, prevalence of CRE colonisation increased over time, with an increase in average of 4.2% per day and mean CRE colonisation rates increased from 13% on admission to 89% after 15 days of hospitalisation (Tran et al., 2019).

Collectively these data show the increasing relevance and resistance of *Acinetobacter* in hospital acquired infections and the newly emerged and rapidly spreading severe clinical problem of carbapenem resistance among *Enterobacteriaceae*, often necessitating use of colistin (an expensive, hard to administer, suboptimal drug with many side effects). However, a limitation of most of these studies was that they were lacking clinical linkage between these isolates and patient centered outcomes.

Given the limitations of the available HAI and VAP data in Vietnam, it is important to have clinical cohort studies to provide accurate, prospective data on the aetiologies of HAI and VAP, on the associated risk factors and also the impact of HAI and VAP on length of ICU stay, cost and mortality. Integration of such data collection within the proposed clinical trials, of antibiotic stewardship and VAP prevention interventions, will contribute to the establishment of evidence based, locally appropriate, recommendations for HAI/VAP prevention, antibiotic use and infection control program in Vietnam.

1.5. Rationale for the project

Infection control procedures and measures to prevent HAI and the transmission of resistant pathogens have been implemented in response to the threat of antimicrobial

resistance. A comprehensive programme (Storr et al., 2017) including strategies to limit transmission of pathogens between patients, control environment related factors for HAIs, use of appropriate treatment and optimal antibiotic prescribing, minimizing invasive intervention and implementation of surveillance and education has been implemented to varying degrees in different settings. Transmission of pathogens through health care worker's hands has often been a target for intervention, however compliance to hand hygiene and other precautions is low among healthcare workers (Erasmus et al., 2010) and poor hand hygiene practice has been observed in patient contact (Johansson et al., 2011) and may not be highly effective in reducing ESBL-producing *Enterobacteriaceae* transmission (Schultsz et al., 2013). Other measures, recommended for routine use in intensive care to prevent VAP by bodies such as Infectious Diseases Society of America, and Spanish Societies of Intensive Care Medicine (Kalil et al., 2016, Alvarez Lerma et al., 2014), such as semi-recumbent body position did not show benefit in preventing ventilator associated pneumonia in Vietnamese patients with severe tetanus (Loan et al., 2012). Additionally, some interventions are probably not appropriate in limited resource settings, due to high costs and nursing requirements or the high levels of resistance already present (Gaillot et al., 2011). Thus, tailored interventions that are appropriate to the setting are required.

High antibiotic consumption, lack of appropriate evidence-based interventions for infection prevention, and the high level of antimicrobial resistance underlie the importance of antimicrobial stewardship in Vietnamese hospitals. The research questions contained within this PhD project were developed to address the burden of antimicrobial resistance in Vietnamese hospitals, and in particular on the intensive care and VAP:

1. What is the current use of antimicrobials in public hospitals in Vietnam?
2. What is the direct cost of management of VARI in Vietnam?
3. Can Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) improve the proportion of patients treated with optimal therapy within 24 hours of positive culture, thereby contributing to Antimicrobial Stewardship?
4. What is the incidence of hospital-acquired bloodstream infections, risk factors for their acquisition and their microbiological aetiology & susceptibility in 3 ICUs in Vietnam?

5. What is the effectiveness of continuous endotracheal cuff pressure control for the prevention of ventilator associated respiratory infections and reduction of antibiotic use in ICU?

In 2013, Vietnam released a national action plan on combatting antimicrobial resistance (AMR) in the period from 2013 – 2020 (Ministry of Health, 2013). This was the first ever national plan to address the burden of AMR in the country by focussing on:

1) raising awareness of community and health workers on antimicrobial resistance

2) strengthening and improving national surveillance system on the use of antimicrobial and AMR

3) ensuring adequate supply of quality medicines

4) promoting proper safe use of antimicrobials

5) promoting infection control

6) promoting proper safe antimicrobial use in livestock, poultry, aquaculture and cultivation.

My PhD project placed an emphasis on investigating the antimicrobial use in the country, providing the insights on the burden of hospital acquired infections (HAI) in term of morbidities and economic burden, focusing on feasible technological intervention efforts to reduce antimicrobials and prevent ventilator associated respiratory infection (VARI), the most common type of HAI in critical care units. My thesis focused on model evidence-based strategy with the aims of measuring the current situation of HAIs and seeking feasible approaches to address the burden of antimicrobial resistance in Vietnamese hospitals that are fit for purpose to the specific context in Vietnam, a lower middle-income country. These aims are in line with and contribute bullet points 2, 3 and 5 of the National Action Plan.

As antimicrobial drug resistance is driven by all use of antimicrobials, whether appropriate or inappropriate, the starting point of my PhD thesis was to describe the use and cost of antimicrobials in Vietnam. Additionally, the data of cost of antimicrobials, especially the last resort antibacterials for treating multidrug resistant patients is used to identify the economical burden and morbidity of VARI in critical care units in Vietnam. To

address the burden of antimicrobial drug resistance in ICUs in Vietnam by focusing on VARI, the most common type of HAIs, I examined two potential strategies to reduce the antimicrobial use, including the early pathogen identification and prevention of the aspiration of contaminated oropharyngeal secretions. The thesis included 4 separate studies with primary objectives as below:

- Study 1: Price and use of antimicrobials in hospitals in Vietnam
  - To describe the price and use of antimicrobials in hospital in Vietnam
- Study 2: Excess direct hospital cost of treating adult patients with ventilator associated respiratory infection (VARI) in Vietnam
  - To estimate the base case cost of treating of VARI and annual total excess direct hospital cost of managing VARI in Vietnam
- Study 3: MALDITOF Versus Routine Clinical Microbiology for Identifying Pathogens: a Randomized Diagnostic Trial (MALDITOF).
  - To compare the proportion of patients on optimal antibiotic treatment within 24 hours of positive culture (first growth of an eligible specimen) in patients whose samples are processed using standard laboratory techniques and those whose are processed using MALDITOF.
- Study 4: The VARI-prevent Trial: A randomised trial of continuous endotracheal cuff pressure control compared with intermittent manual pressure checks for the prevention of ventilator associated respiratory infections (VARI).
  - To determine the incidence of hospital acquired bloodstream infections in intubated patients admitted to ICUs in Vietnam
  - To determine whether automated cuff pressure control results in a reduction in the proportion of patients developing ventilator associated respiratory infections compared with routine care.

Chapter 2: **Methodology**

This chapter described the methods and materials for 3 subsequent chapters in this thesis. It contains 4 individual study designs:

i) a descriptive study of antimicrobial price and use in public hospitals in Vietnam (Chapter 3),

ii) a modelling study of excess cost of ventilator associated respiratory infection (VARI) in critical care units in Vietnam (relating to Chapter 4);

iii) a randomised controlled trial of matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) versus routine clinical microbiology for identifying pathogens (relating to Chapter 5) and

iv) a randomised controlled trial of continuous versus intermittent endotracheal cuff pressure control for the prevention of ventilator associated respiratory infections (relating to Chapter 6 and Chapter 7).

2.1. Method for Chapter 3: Price and use of antimicrobials in an emerging pharmaceuticals market in Vietnam

2.1.1. Objective

This study describes antimicrobial usage, expenditure and cost in public hospitals in Vietnam.

2.1.2. Study design

The process of bidding for contracts to supply medication to public health facilities follows Vietnam government guidance (Ministry of Health, 2016). Health facilities are reponsible for preparing an invitation to tender for medications based on their projected demand (usually largely based on the quantity used in the preceding year). The health facility is required to ensure the consumption of at least 80% of each medication purchased (Ministry of Health, 2016).

In this study, antimicrobial consumption in public hospitals in Vietnam was estimated using data from successful tenders for the purchase of antimicrobials for 52/63 Provincial

Departments of Health, 23 secondary hospitals and 7 primary hospitals (outside the 52 provincial departments) throughout Vietnam. As of December 2017, Vietnam had 13,583 public healthcare facilities, including 1,085 hospitals with 308,400 patient beds, 579 regional clinics and 11,830 medical service units in communes, wards, offices and state- or privately-owned enterprises (Vietnam Ministry of Health, 2016, General Statistics Office, 2018). In the private sectors, there were 219 private hospitals in the country by 2018. The country's healthcare system is divided into four technical categories: tertiary hospitals (under administrative control of or appointed by the Ministry of Health), secondary hospitals (under the Provincial Departments of Health (DoH) and catering to and receiving referrals from the province population), primary hospitals (district hospitals under Provincial DoH, catering to and receiving referrals from the district population, commune health stations), and commune health stations or medical service units) (Vietnam Ministry of Health, 2013, Dat et al., 2017a). Currently there are 75 tertiary hospitals, 491 secondary hospitals, 514 primary hospitals and 5 unclassified hospitals (Vietnam Ministry of Health, 2016).

2.1.3. Data resources

Data on the cost of antimicrobials in Vietnam were taken from the successful tenders for medicines in 2018 for hospitals and provincial Departments of Health (DoH) in Vietnam as published on the website of the Drug Administration of Vietnam website (Drug Administration of Vietnam, 2018). DoHs will tender for the supply of medication to the secondary and primary hospitals under their jurisdiction. The bid winning tenders from provincial DoH may cover all or only some of the primary and/or secondary hospitals within that province and the data on precisely which hospitals, or the breakdown by type within each bid, were not available. I collected all available bid-winning tenders in 2018 for 23 secondary hospitals, 7 primary hospitals and 52 provincial departments of health. The data for each tender included the name of the active ingredient, trademarks, strength, dosage and package, route of administration, registration identification, manufacturer, country of origin of manufacturer, measuring unit, bid quantity, unit price, total value, bidders, offerors and brand name/generic name (Ministry of Health, 2016). The list of antimicrobials in our analysis excluded antimicrobial medications which are nationally procured, such as those for the treatment of HIV, influenza, tuberculosis and malaria.

2.1.4. Estimation of antimicrobial procurement and the cost of antimicrobials

All antimicrobials for systemic use were included in the analysis and classified using the Anatomical Therapeutic Chemical (ATC) Index with Defined Daily Doses (DDDs) 2018 (WHO Collaborating Centre for Drug Statistics Methodology, 2018). The DDD is recommended by WHO as a measurement unit of drug consumption (WHO International Working Group for DrugStatistics Methodology et al., 2003). It is the average maintenance dose of a drug per day for a 70 kg adult for its main indication. It provides an estimate and comparison of drug consumption between population groups and is widely used in pharmacoeconomical studies. The DDD for a given drug is assigned by ATC/DDD classification with a unique code and may be different for the routes of administration (oral and parenteral) of the same drug when there is a substantially difference of bioavailability. It is neither defined for topical products nor available for all drug combinations (WHO Collaborating Centre for Drug Statistics Methodology, 2017). Antibacterials were further classified by AWaRe categories following the 2017 revision of the WHO Model List of Essential Medicines (see Table 2-1) (World Health Organization, 2017c).

Table 2-1. AWaRe antibacterial classification

ACCESS group antibacterials		
amoxicillin	amikacin	cefixime*
amoxicillin + clavulanic acid	chloramphenicol	cefotaxime*
ampicillin	clindamycin	ceftriaxone*
benzathine benzylpenicillin	doxycycline	piperacillin + tazobactam*
benzylpenicillin	gentamicin	meropenem*
cefalexin	metronidazole	azithromycin*
cefazolin	nitrofurantoin	ciprofloxacin*
cloxacillin	spectinomycin	clarithromycin*
phenoxymethylpenicillin	sulfamethoxazole + trimethoprim	vancomycin *
procaine benzyl penicillin		
WATCH group antibacterials		
Quinolones and fluoroquinolones		
3rd-generation cephalosporins (with or without beta-lactamase inhibitor)		
Macrolides		
Glycopeptides		
Antipseudomonal penicillins + beta-lactamase inhibitor		

Carbapenems	
Penems	
RESERVE group antibacterials	
Aztreonam	Fosfomycin (IV)
4th generation cephalosporins	Oxazolidinones
5th generation cephalosporins	Tigecycline
Polymyxins	Daptomycin

*Antibacterials that are recommended as first or second choice treatments for a few, specific indications but also in the WATCH group (Sharland et al., 2018). Source: WHO model list of essential medicines, 20th list (March 2017, amended August 2017).

I calculated the total DDD procured for an antimicrobial by multiplying the total procured at each dose-route of administration for this drug by the DDD conversion factor for the corresponding dose-route of administration. The average cost per DDD of an antimicrobial drug was calculated by dividing cost for that drug by the total number of DDD. The high/low ratio was used to compare the difference between the highest unit price and the lowest price of one DDD of each antimicrobial across all the tenders. All costs were converted from VND to US$ according to the annual average official exchange rates of the World Bank in 2017 (US$1 = 22,370.09 VND) (The World Bank Group, 2019). The average price of antimicrobials per DDD, the share of antimicrobials cost in the hospital drug budget and the number of DDD are used to compare in different levels of hospitals. Pareto chart (ABC analysis) was used to examine the consumption of antimicrobials and expenditures for procurement (Embrey and Management Sciences for Health (Firm), 2013). The ratio of the highest to the lowest price of antimicrobials per DDD (high/low (H/L) ratio) was calculated to report the variation of antimicrobials price (Management Sciences for Health (MSH), 2015). I used Spearman correlation coefficient to assess the association between variation of antimicrobials prices (high/low ratio) and number of manufacturers. Descriptive statistics was performed using Microsoft Excel (Office 365, version 1909, Microsoft Corporation, Redmond, Washington,).

2.2. Method for Chapter 4: Excess direct hospital cost of treating ventilator associated respiratory infection (VARI) in Vietnam

2.2.1. Objectives

This study aims to estimate the total excess annual cost to the National Health Service of the management of VARI in critical care units in tertiary hospitals throughout Vietnam.

2.2.2. Study design

I estimated the excess cost of VARI in Vietnam using a incidence-based cost-of-illness approach (Tarricone, 2006) from a healthcare sector perspective which included all costs covered by the governmental health insurance and co-paid by patients. I did not distinguish between ventilator associated pneumonia (VAP) and ventilator associated tracheobronchitis (VAT) as the pathogenesis and bacterial aetiology of the two entities are essentially the same and both are treated for a similar duration in Vietnam. A Vietnamese study analysing the costs of VAP and other VARI found little difference when similar patient groups were looked at (Phu et al., 2017). All direct medical costs were estimated as the product of number of services and their unit cost. Micro-costing methodology was used to cost the management of VARI which was composed of cost components of diagnostics, hospital stays and antimicrobials. By using this method, the direct costs were estimated as the product of number of services used and their unit cost.

2.2.3. Data resources

Based on my previous study in 2015, I used data of 25 provincial hospitals and 103 district hospitals in Vietnam to derive the number of hospital beds with access to mechanical ventilation in Vietnam and the proportion of these ventilators in frequent use (Dat et al., 2017a). In this survey critical care beds accounted for a median of 4% (IQR 3-5%) of the provincial hospital beds and for 9% (IQR 5-12%) of the district hospital beds. This corresponded to median critical care unit (CCU) sizes of 26 beds (IQR 17-33) and 12 beds (IQR 8-19) in provincial and district levels respectively. All provincial CCUs had ventilators with a median number of mechanical ventilators of 11 (IQR 6-16 or 0.4 per actual critical care bed (IQR 0.26–0.55)). However, only 73.6% of district hospitals had ventilators with a median of 0.08 (IQR 0.00-0.17) ventilators per actual critical care bed. Whilst most provincial hospitals have adequate capacity to deliver critical care services, district hospitals have limited capability in this respect, typically only ventilating patients prior to

transfer to a higher level of care (Dat et al., 2017a). I therefore include only central and provincial hospitals in modelling the burden of VARI.

The prevalence and associated increased length of CCU stay of VARI were extracted from literature review (Table 2-2). Aetiology and susceptibility of VARI causing pathogens were extracted from a previous point-prevalence surveys in 15 hospitals in Vietnam (Table 2-3) (Phu et al., 2016). Data on the unit costs of healthcare services in Vietnamese CCUs were taken from documents issued by the ministry of health regarding costs for medical services among hospitals at the same level across the country (Ministry of Health and Ministry of Finance, 2015, Ministry of Health, 2017). Costs for these services are inclusive of certain direct expenses (i.e. consumable materials, substitute items), expenses for electricity, water, fuel, waste, treatment, maintenance and replacement of equipment and employee allowances and salaries (Ministry of Health and Ministry of Finance, 2015). Assumptions for the costing model are shown in Table 2-2.

Table 2-2. Assumptions of a costing model for ventilator associated respiratory infection (VARI) in Vietnam

Assumption	Value (range)	Source(s)
Number of critical care units (hospitals)	577	(Vietnam Ministry of Health, 2016)
Number of ventilators per critical care units	11 (6-16)	(Dat et al., 2017a)
Proportion of patients with intubation ≥ 48 hours	88.9% (87.6%-90.2%)	(Ha et al., 2015)
Incidence density of VARI per 1000 ventilation days	21.7 (17.7 – 26.5)	(Phu et al., 2017)
Percentage of ventilators in frequent use	77% (± 10%)	(Vietnam Medical Services Administration, 2015)
The de-escalation rate	23% (21.3%-68%)	(Alvarez-Lerma et al., 2006)
Extra CCU length of stay for each episode of VARI (days)	12 (± 2)	(Phu et al., 2017)
Extra duration of antibiotics (days)	12 (7-14)	(Phu et al., 2017, Kalil et al., 2016)
Excess duration of ventilation (days)	12 (± 2)	(Phu et al., 2017)
Range of aetiologies of VARI	See Table 2-3	(Phu et al., 2016)

Data on the cost of antimicrobials in Vietnam was taken from the 2017 medication bid-winning results of national and provincial hospitals in Vietnam as published on the Drug Administration of Vietnam website (Drug Administration of Vietnam, 2018). The bid-winning price reflects the diversity in pricing of antimicrobials across 93 national and provincial hospitals in 27 provinces in Vietnam. The average price of antimicrobials was applied for the model.

I developed a decision tree for antimicrobial treatment of VARI based on local data on the microbiological aetiology of VARI and internationally recognised recommendations for antimicrobial treatment (Torres et al., 2017, Kalil et al., 2016) which is aligned with the national guidelines issued by the ministry of health for use in Vietnamese hospitals (Ministry of Health, 2015). The local data for aetiology and associated antibiotic susceptibility was derived from a previous point-prevalence survey in Vietnam (Phu et al., 2016) (Table 2-3) and used in conjunction with the guidelines of the Infectious Diseases Society of America and the American Thoracic Society (or where the treatment recommendation was not available for specific pathogens, the Sanford guide to antimicrobial therapy) to determine the antimicrobial treatment administered (David N. Gilbert et al., 2017, Kalil et al., 2016) (Figure 2-1).

Table 2-3. Assumed aetiology of ventilator associated respiratory infections

Name of microorganisms	Proportion
No identification of pathogens	61.5%
Identification of pathogens	38.5%
Enterobacteriaceae	6.7%
Escherichia coli	1.3%
Non-susceptible to the 3GCs and CARB	5.4%
Susceptible to the 3GCs and non-susceptible to CARB	54.1%
Susceptible to 3GCs and CARB	40.5%
Klebsiella pneumoniae	4.5%
Non-susceptible to the 3GCs and CARB	14.9%
Susceptible to the 3GCs and non-susceptible to CARB	56.8%
Susceptible to 3GCs and CARB	28.4%
Klebsiella spp.	2.9%
Non-susceptible to the 3GCs and CARB	5.7%
Susceptible to the 3GCs and non-susceptible to CARB	62.3%

Name of microorganisms	Proportion
Susceptible to 3GCs and CARB	30.1%
Other E*nterobacteriaceae*	3.3%
Non-susceptible to the 3GCs and CARB	12.5%
Susceptible to the 3GCs and non-susceptible to CARB	59.4%
Susceptible to 3GCs and CARB	28.1%
Non-fermenters	21.8%
Acinetobacter baumannii	9.9%
Non-susceptible to CARB	89.2%
Susceptible to CARB	10.8%
Pseudomonas aeruginosa	6%
Non-susceptible to the 3GCs and CARB	55.7%
Susceptible to the 3GCs and non-susceptible to CARB	44.3%
Susceptible to 3GCs and CARB	
Acinetobacter spp.	3%
Non-susceptible to CARB	89.2%
Susceptible to CARB	10.8%
Other Gram-negative bacilli	2.9%
Gram-positive bacteria	3.8%
Staphylococcus aureus	1.8%
Methicillin-resistant *Staphylococcus aureus* (MRSA)	75.7%
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	24.3%
Staphylococcus spp.	0.7%
Non-susceptible to oxacillin	36.4%
Susceptible to oxacillin	63.6%
Enterococcus spp.	0.7%
Vancomycin-resistant Enterococcus (VRE)	57%
Vancomycin-susceptible Enterococcus (VSE)	43.0%
Streptococcus spp.	0.6%
Fungi (*Candida spp.*)	0.9%

3GC: Third-generation cephalosporins; CARB: carbapenem

Adapted from Phu et al (Phu et al., 2016).

The cost of VARI included the costs of initial empirical and targeted therapy regimens. The initial broad-spectrum empirical therapy was modelled to cover *P. aeruginosa* and

ESBL-producing organisms, *Acinetobacter* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA) for the setting with high rates of multidrug resistant pathogens (Torres et al., 2017, Kalil et al., 2016). The empirical therapy was assumed to last for 3 days and followed by the targeted therapy (Figure 2-1).

Analysis was limited to adult patients because of limited data in the paediatric critical care population concerning the aetiology of VARI, provision and utilisation of critical care beds and difficulties accounting for the spectrum of ages to adjust antimicrobial doses.

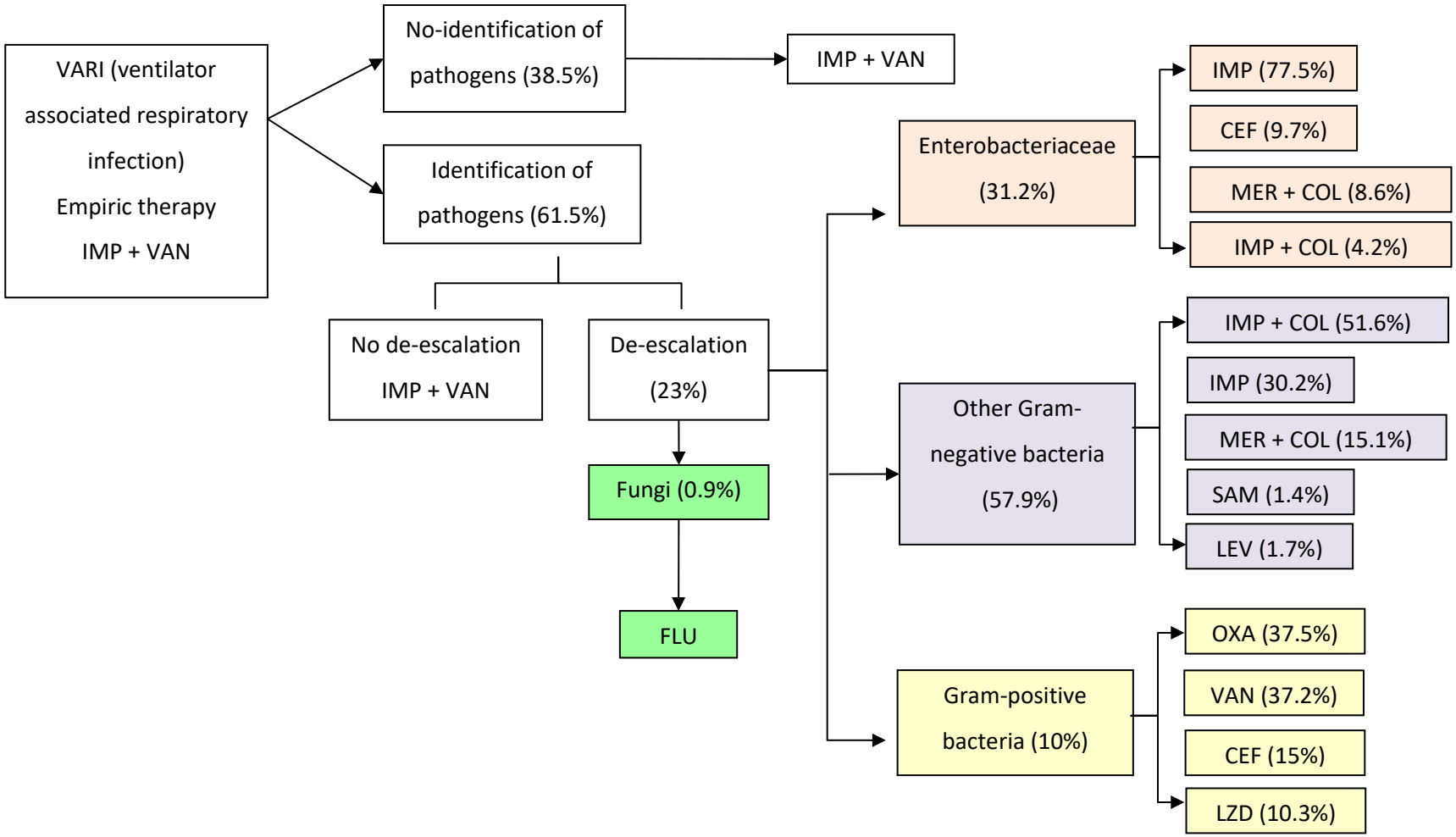


Figure 2-1. Decision tree for antibiotic treatment for ventilator associated respiratory infection (VARI).

CEF, ceftriaxone; COL, colistin; FLU, fluconazole; IMP, imipenem-cilastatin; LEV, levofloxacin; LZD, linezolid; MER, meropenem; OXA, oxacillin; SAM, ampicillin-sulbactam; VAN, vancomycin.

2.2.4. Estimation of the cost of VARI management and number of VARI episodes

Estimates of the cost of VARI episodes were based on the excess direct medical costs of diagnostics, hospital stay, ventilation and antimicrobial treatment. All costs were at 2017 prices and presented in US dollars (US$) based on the official exchange rate of 2016 (1 US$ = 21,935 Vietnam Dong) (World Bank, 2018b). The diagnostics for VARI included investigations for VARI (complete blood count, arterial blood gas, bedside chest radiography, endotracheal aspirate smear and culture, blood culture and antibiotic susceptibility when the pathogen was identified) (Torres et al., 2017, Kalil et al., 2016). These investigations are performed routinely in referral hospitals in Vietnam and recommended by the ministry of health for critical care units. All investigations were assumed to be performed at the time of VARI suspicion. The excess cost of hospital stay and ventilation was calculated by the quantity of extra days multiplied by their daily prices (Table 2-3 and Table 2-4).

The antimicrobials cost per VARI episode was composed of the cost of three days of empirical therapy with imipenem-cilastatin (IMP) and vancomycin (VAN) and the cost of targeted therapy. For VARI without pathogen identification, we assumed that the empirical treatment continued for the whole course of VARI treatment in n days:

$$C_{\text{emperical antimicrobials}} = n \times (C_{IMP}+C_{VAN})$$

For cases with pathogen identification, in the absence of local data, we assumed that the de-escalation was 23% as reported in a prospective Spanish observational study (Alvarez-Lerma et al., 2006) and the empirical therapy remained unchanged in 77% of VAP patients. We calculated the proportion of antimicrobial regimens corresponding to the frequency of pathogenic isolates in patients with antimicrobial de-escalation therapy. The per-episode antimicrobial cost of microbiologically confirmed VARI cases was calculated as follows:

$$C_{\text{targeted antimicrobials}} = 3\times(C_{IMP}+C_{VAN}) + \sum (n\text{-}3)C_k P_k$$

In which, n is the antimicrobial therapy duration (days), k is the antimicrobials regimen, C and P is the daily cost and proportion of usage of the specific regimens.

Table 2-4. Costs associated with management of ventilator associated respiratory infection (VARI) in Vietnam

Item	Average unit cost (min-max) in USD	Notes
Cost for hospital beds	US$ 21.87 (30.98-12.77)	Daily cost
Cost for mechanical ventilation	US$ 22.35 (24.39-20.31)	Daily cost
Cost for diagnostics of VARI		
Complete blood cells	US$ 3.09 (1.46-4.71)	1 time
Air blood gas	US$ 9.42 (9.15-9.70)	1 time
Bedside chest X-ray	US$ 2.91 (2.65-3.16)	1 time
Blood cultures	US$ 11.14 (9.15-13.13)	1 time
Endotracheal aspirate culture	US$ 11.14 (9.15-13.13)	1 time
Endotracheal aspirate smear	US$ 2.80 (2.61-3.00)	1 time
Qualitative antibiotic susceptibility	US$ 7.62 (7.09-8.14)	If the pathogen is identified
C-reactive protein (CRP)	US$ 2.36 (2.29-2.42)	1 time
Cost for antimicrobial treatment		
Linezolid 300 mg	US$ 25.39 (8.62-42.16)	600 mg IV q12h
Meropenem 1 g	US$ 19.29 (3.17-35.41)	1 g IV q8h
Colistimethate Sodium1 MIU	US$ 14.05 (10.48-17.62)	5 mg/kg IV × 1 (loading dose) followed by 2.5 mg × (1.5 × CrCl + 30) IV q12h
Fluconazole 200 mg	US$ 11.65 (6.56-16.74)	400 mg q12h
Imipenem + cilastatin 500/500 mg	US$ 9.46 (2.61-16.31)	500 mg IV q6h
Levofloxacin 500 mg	US$ 7.75 (1.19-14.32)	750 mg IV q24h
Vancomycin 1 g	US$ 4.16 (0.33-7.99)	15 mg/kg IV q8–12h
Ceftriaxone 1g	US$ 3.92 (2.47-5.37)	1 gm IV q24h
Ampicillin sulbactam 1g/0.5 g	US$ 1.88 (0.44-3.33)	3 g IV q6hr
Ceftazidime 1g	US$ 1.69 (0.48-2.91)	2 g IV q8h
Oxacillin 1g	US$ 1.25 (0.65-1.85)	2 gm IV q4h

The country's annual number of VARI episodes was estimated from the incidence density of VARI (in cases per 1000 ventilation days) multiplied by the total number of ventilators in frequent use in all critical care units and the proportion of patients intubated for more than 48 hours (see Table 2-2).

2.2.5. Sensitivity analysis

A one-way sensitivity analysis of the total cost of VARI treatment was performed by varying the country capacity for mechanical ventilation, incidence density of VARI, cost of diagnostics, critical care stay and antimicrobials, and duration of antimicrobial therapy. We performed one-way sensitivity analyses in which each factor was individually varied within the assumed plausible ranges as described above whilst all other factors remained at the central values.

Among variables included in the costing model, the number of critical care units was considered as fixed value whilst the proportion of patients with intubation ≥ 48 hours and percentage of ventilators in frequent use were varied by ±10% from the average values. We varied the number of ventilators per critical care unit by the interquartile range as reported in the previous study in Vietnam (Dat et al., 2017a). Because the antimicrobial therapy de-escalation rate was not reported in Vietnam, we varied this rate by the values from the prospective study of 24 intensive care units in Spain (Alvarez-Lerma et al., 2006). Varying the incidence density of VARI was performed with the additional analysis of data from a previous prospective observational study in 4 CCUs in Vietnam (Phu et al., 2017). The extra duration of antimicrobial therapy was varied between 7-14 days to cover 2 common antimicrobials regimens of 7-8 days and 10-14 days (Torres et al., 2017). The excess CCU length of stay and duration of ventilation was varied by ±2 days from the difference of length of stay (12 days) between patients with VAP and no VAP in a meta-analysis (of studies largely in high income settings) and a prospective study in Vietnam (Melsen et al., 2013, Phu et al., 2017). The range of cost values is shown in the Table 2-2.

2.3. Method for Chapter 5: Impact of MALDITOF diagnostic pathway on optimal antimicrobial therapy

2.3.1. Objectives

The primary objective of this study was to determine if MALDITOF-MS can improve the prescribing of antimicrobial therapy in patients with culture-confirmed infection. Secondary objectives were to establish the impact of MALDITOF-MS on patient centred outcomes and cost of treatment during hospitalisation.

2.3.2. Study design

This was a randomised controlled trial conducted in 2 tertiary hospitals in Vietnam that specialise in infectious diseases: the National Hospital for Tropical Diseases (NHTD) in Hanoi and the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City. Both have ISO15189 accredited microbiology laboratories. This trial compared the Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS – the intervention arm) to the current standard microbiological methods (control arm) for identifying pathogens.

2.3.3. Study population

Recruitment was performed between January and December 2015 among patients with at least one pathogen cultured from an eligible sample. Eligible samples were blood cultures or aspirates from sterile compartments ('other' samples - cerebrospinal fluid (CSF), deep abscesses, joint fluid, peritoneal fluid, pleural fluid or deep tissue biopsies). All subsequent samples from sterile compartments from a patient with an eligible sample were to be analysed using the same diagnostic pathway (MALDITOF-MS or standard microbiological methods). A patient was not eligible for recruitment if, at the time of randomisation, they had already had an eligible sample processed during the same hospital admission.

2.3.4. Study endpoints

The primary endpoint was the proportion of patients on optimal antimicrobial treatment (OAT) within 24 hours of positive culture (first observed growth of an eligible specimen). Optimal antibiotic treatment was defined as an antibiotic treatment lasting for at least 48 hours since positive culture, targeted to the identified pathogen and later found to cover the organisms antimicrobial resistance profile, while avoiding unnecessary broad-spectrum antibiotics (e.g. avoid carbapenems or multiple agents where other agents or single agents would provide sufficient coverage). Examples of optimal antibiotic treatment are for example: stopping vancomycin treatment when a *Klebsiella spp* is identified or starting melioidosis specific treatment in case *Burkholderia pseudomallei* is identified. In case another invasive pathogen was identified within 48 hours of administering the optimal treatment that was not covered in the antibiotic spectrum, then the treatment is not considered optimal.

The decision on whether therapy was optimal within 24 hours was made by an independent review committee, blinded to the allocated diagnostic arm. The committee individually reviewed a standard subset of data collected in the case report form (CRF) which included the admission and discharge diagnosis, the antibiotics used over the admission and the full microbiology results (including susceptibility testing) for that episode. These data, which were free of any evidence of the allocated arm, were scrutinised in retrospect for all cases to determine the following: OAT at 24 hours and 48 hours or at any point during hospital stay. If antibiotic therapy was not optimal at 24 hours, the reason for this was grouped into: organism not covered; therapy too broad; and 'other'). Discrepancies between reviewers were resolved by consensus using international guidelines where available. Where review of the individual clinical scenario and the organism isolated led the independent review committee to feel that the microbiological result should not be the sole focus of antimicrobial therapy, this was recorded as 'unclassified'.

Secondary 'patient-level' endpoints were: OAT within 48 hours of positive culture, the defined daily dose (DDD) of antibiotics prescribed to patients (calculated by summing all the defined daily doses of antibiotics used from enrolment to discharge), the total duration of antimicrobial treatment (calculated by summing each day when any antibiotic was given from enrolment to discharge), hospital and ICU length of stay and clinical outcome (divided into death, palliative discharge (a common practice in Vietnam when a patient was discharged home for palliative care with the expectation of an early death), survived with sequelae and recovered) and hospital care (in US Dollars derived from the hospital bill at the 2018 exchange rate US$1 = 21,935 Vietnam Dong (World Bank, 2018b)) . Patients with meningitis due to *Cryptococcus neoformans* had the costs of their care was supported by a clinical trial over the study period and consequently costs for these patients were not included (Beardsley et al., 2016).

Secondary 'specimen-level' endpoints included the time from specimen collection and first growth from an eligible specimen to OAT and to discharge.

2.3.5. Sample size

We expected that with the rapid results produced by the MALDITOF-MS system, doctors would be able to administer optimal antibiotic treatment more rapidly. Specifically, we expected an increase in the proportion of patients on optimal antibiotic treatment

within 24 hours from first growth of an eligible specimen from 40% (standard diagnostics) to 60% (MALDITOF-MS) (Vlek et al., 2012). To detect such an increase with 90% power at the 2-sided significance level of 5%, a total sample size of 260 eligible specimens is required. To have sufficient power for a subgroup analysis of the trial in positive blood cultures for each hospital separately and accounting for some incomplete data, the trial was to continue enrolment at both hospitals until the slower recruiting hospital reached enrolled 280 blood cultures as first specimen. We aimed to enrol all eligible specimens (blood or other) from both hospitals during that time frame but do not explicitly control the number of 'other' specimens.

Based on data from NHTD and HTD we predicted we would be able to finish recruitment within one year. During a recent 12-month period, NHTD had approximately 282 positive blood cultures, and in the same period HTD had approximately 500 positive blood cultures. In the same period, we expected to be able to enrol ~150 other specimens at NHTD and ~200 at HTD.

Assuming that the total sample size includes data from approximately 1000 subjects, we would have 89% power to detect an increase in the primary endpoint from 40% (routine diagnostics or controls) to 50% (MALDITOF- MS) in the overall analysis of all specimens from both hospitals. Moreover, we were also be able to detect an increase from 40% to 60% in the subgroup of other specimens from both hospitals with a power of >90%.

2.3.6. Randomisation procedure

When an eligible specimen from a patient showed growth of a pathogen, the diagnostic pathway was randomized to either MALDITOF-MS or routine microbiological diagnostics with a 1:1 allocation ratio and stratification by hospital and specimen type (blood vs. other). Isolates grown from all eligible specimens of the same patient were assigned to the same diagnostic pathway as the first randomized specimen of that patient.

Allocation to diagnostic pathway was assigned by a web-based randomization program using a random variable block length of 4 or 6. When a pathogen was isolated from a positive eligible specimen, the laboratory technician logged onto the secure randomization program and entered the patient and specimen code. The random diagnostic pathway allocation then was generated, informed to the laboratory technician and logged in the study database. In the case of multiple specimens with pathogen growth for a single

patient, the unique patient code triggered the randomization program to generate the same diagnostic pathway allocation as the previous sample(s).

2.3.7. Intervention

Clinical specimens were collected according to routine practice. Briefly, blood culture bottles (aerobic) were incubated for up to five days in an automated culture system (Bactec, Becton-Dickinson, USA). Other samples were incubated on media allowing growth of aerobic, anaerobic and fastidious organisms and checked daily for growth. For the MALDITOF-MS arm, blood culture media or colonies from plates were sub-cultured onto blood agar until growth could be observed. Colonies were then analysed by MALDITOF-MS analysis twice per day. In the routine arm, methods for identification included: Gram stain, API test strips (bioMérieux), VITEK2 system (bioMérieux), and other biochemical and microbiological tests (e.g. oxidase, optochin sensitivity etc). Samples that yielded organisms that were not considered pathogens (e.g. coagulase-negative staphylococci, diphtheroids, isolated growth of viridans group streptococci) were not included in analysis. Treating physicians were not explicitly told of the allocated diagnostic pathway for the patients, but could have made deductions from the speed of results for that patient.

2.3.8. Study assessment

Both clinical and microbiological data for participants were collected prospectively, recorded onto a case Record Form (CRF), and checked for accuracy by research staff. These data included all antibiotic and antifungal medications prescribed, hospital related costs to the patient or their insurance incurred (through the hospital bill) and outcome. At least 24 hours following the delivery of the written report on the pathogen identity to the ward, a member of the research team discussed with the clinical team whether the results in the report had changed management and if not, why not. Data were then anonymised and entered to an online, secure electronic database by single entry method.

No other changes were made to the routine hospital procedures for the communication of microbiological culture results between the laboratories and the clinical teams. This involved telephoning of positive culture results where staffing permitted it and provision of written reports to the wards (no computerised reporting system was available at either site during the study period).

Table 2-5. Schedule of enrolment, interventions, and assessments in the MALDITOF-MS trial

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	*First growth of pathogen*		*Pathogen identification*	*24 hours after positive culture*	*48 hours after positive culture*	*Hospital discharge*
ENROLMENT:						
Eligibility screen	X					
Informed consent	X					
Randomisation		X				
INTERVENTIONS:						
MALDITOF-MS		X	X			
Standard microbiological diagnostics (controls)		X	X			
ASSESSMENTS:						
Demographic data	X					
Type of specimen	X					
Pathogen isolates			X			
Antimicrobial susceptibility			X			
Date and time of antimicrobial switches	X	X	X	X	X	
Prescribed antimicrobials	X	X	X	X	X	X
Defined daily dose (DDD)						X
Diagnosis	X					X
Mortality						X
Length of stay						X
Cost of microbiological testing						X
Cost of stay						X
Optimal antibiotic treatment review				X	X	

2.3.9. Data management

Both microbiological and clinical data were recorded onto a CRF and checked for accuracy by the clinical microbiologist on duty. Data was entered to an electronic database (CLIRES) by single entry method. Internal checks of the entered data were performed to look for outliers and errors.

2.3.10. Statistical Analysis

2.3.11. Ethics approval and consent to participate

The study was reviewed and approved by The Ethical Committee in Biomedical Research - Hospital for Tropical Diseases (Approval number 16/HDDD-QD), the Institutional Review Board – National Hospital for Tropical Diseases (Approval number 698/QD-NDTW) and the Oxford Tropical Research Ethics Committee (OXTREC Reference: 55-14) with respect to scientific content and compliance with applicable research and human subject regulations. The trial was registered with Clinicaltrials.gov (NCT02306330).

In NHTD, patients admitted to the participating hospitals received an information sheet with details about the study. This sheet informed patients of the purpose and procedures of the study as well as their right to refuse participation and how to get more information or withdraw from the study. Any patient who requested not to be included in the study had their specimens labelled accordingly and diagnosis proceeded in the standard way. At HTD, in accordance with the Ethical Committee approval at that site, patients (or their legal representatives) who had growth in an eligible sample were seen by study staff, fully informed of the study, and asked for written consent before participation. Only those who provided consent were recruited.

2.3.12. Statistical Analysis

For analysis of the primary outcome, we used a logistic regression model of the primary endpoint depending on the treatment groups, with additional adjustment for the first specimen type (blood vs. other) and study site. As a conservative measure, subjects with an ‘unclassified’ primary endpoint were labelled as ‘non-optimal’, as were subjects that were discharged or died within 24 hours unless OAT had been started before death/discharge. We planned the following subgroup analyses: specimen type (blood/other); study site (hospital); type of pathogen (fungi, Gram positive or negative bacteria); final diagnosis of meningitis and non-meningitis. These subgroup analyses and the secondary endpoint of OAT within 48 hours of positive culture were performed in the same way as for the whole population. Comparisons of the secondary endpoints of total antibiotics prescribed and hospital stay were performed using a linear regression model with treatment as the main covariate and adjustment by specimen type and study site.

Where the outcome was right skewed the endpoint was log-transformed prior to analysis leading to a comparison of geometric rather than arithmetic means. Hospital and ICU length of stay were analysed using Cox proportionate hazards, stratified by specimen type and study site. A summary of these endpoints for survivors only is also provided. Outcome was categorised as death or palliative discharge, survived with sequelae (including intra-hospital transfers other than palliative ones) and recovered. The ordinal outcome was compared between the two diagnostic arms using a proportionate odds cumulative logit model with adjustment by specimen type and study site. A non-prespecified analysis of outcome as a binary variable was also conducted (death and palliative discharge versus other) using a logistic regression model adjusted for specimen type and study site. Time from first growth and collection of sample to OAT were analysed using a standard Cox regression analysis with competing risks (death or discharge without OAT) treated as right-censored at time infinity to indicate that they never received OAT. Time from first growth and sample collection to issue of the pathogen identification report in the laboratory were descriptively summarised by diagnostic arm as median (lower and upper quartiles) with the formal statistical comparison. Analyses were performed using R (Version 3.4.0). P values below 0·05 were considered significant (two-sided).

2.3.13. Funding

The study was funded by the Wellcome Trust Asia Programme grant and the Li Ka Shing Foundation.

2.4. Method for Chapter 6: Hospital acquired blood stream infection in intubated patients in 3 Vietnamese ICUs and Chapter 7: Descriptive analysis of ventilator associated respiratory infections in a randomised controlled trial in the ICUs

2.4.1. Objectives

The objectives of both chapters five and six were met through a single study. These objectives were as follows:

2.4.1.1. Objectives of Chapter 6

The objective of this chapter is to estimate the incidence of hospital acquired bloodstream infection (HABSI) and central line associated bloodstream infection (CLABSI) among intubated patients, the aetiology and risk factors for HABSI of these patients.

2.4.1.2. Objectives of Chapter 7

The primary objective of this study was to determine if continuous endotracheal cuff pressure control (CPC) can reduce the incidence of ventilator associated respiratory infection (VARI) in adults. Secondary aims included establishing whether CPC leads to an increase in proportion of ventilated days in ICU not on antibiotics or a reduction in antibiotic costs, a reduction in the frequency of hospital acquired infection (HAI) as a whole during ICU stay, length of ventilation, length or cost of ICU and hospital stay, as well as any local complications of the endotracheal cuff and mortality at 28 and 90 days post randomisation and at discharge from ICU. In this thesis, I also present a brief data analysis plan and demographics of patients with VARI.

2.4.2. Study design

This was an open-label, randomised controlled trial (RCT) comparing continuous endotracheal cuff pressure control via an automated electronic device, with intermittent manual pressure control for the prevention of VARI in ICU. For the purpose of this study a stand-alone CPC device was be used (Tracoe reference 701) for the intervention. The RCT also provided opportunity to monitor the occurrence of hospital acquired infection prospectively. Although recognition and diagnosis of HAIs and intervention at early stage are expected to impact the mortality, length of stay and treatment cost, it is clear that there are important differences between surveillance definitions and actual clinical definitions. Therefore, the observational study of hospital acquired infections can be integrated in the trial to provide insight on the real-life diagnosis and treatment of HAIs.

2.4.3. Study population

Patients were recruited over an 18-month period from the two ICUs in the Hospital for Tropical Diseases (HTD), the general ICU in Trung Vuong Emergency Hospital (TVH) in Ho Chi Minh City and the National Hospital of Tropical Diseases (NHTD) in Hanoi. HTD and NHTD are referral centres for the management of patients with infectious diseases. Both receive patients with infectious diseases directly from their local populations as well as

transferred from other hospitals in southern and northern Vietnam. TVH is a provincial hospital serving the general medical needs of the local population in HCMC.

Patients were considered eligible for inclusion in the study if they were at least 18 years of age, had been intubated for ≤ 24 hours at the time of randomization (either orally or through tracheostomy) and for active treatment (i.e. the physician caring for patient would prescribe an antibiotic if the patient developed an infection). Exclusion criteria were previous enrolment in the study, having been previously intubated within the last 14 days, known tracheal stenosis, tracheomalacia or stridor that is suspected secondary to physical tracheal injury. Patients were only enrolled following written informed consent from themselves or their legal representatives.

Patients were stratified by clinical diagnosis of tetanus or non-tetanus, as patients with tetanus represent a distinct subgroup: usually they present without significant premorbid disease or systemic inflammatory response, often undergo primary tracheostomy and have a prolonged duration of intubation and ICU stay. Whilst rare in high-income settings, in many countries tetanus patients represent a significant burden of disease with high rates of HAI and high consumption of ICU resources (Thwaites and Farrar, 2003). Thus, any study attempting to address the problem of HAI in this setting also needs to specifically address tetanus without allowing it to dominate the picture. In view of this patients with tetanus were limited to 30% of the total enrolment.

2.4.4. Study endpoints

The primary endpoint was the occurrence of VARI which was defined as Ventilator Associated Pneumonia (VAP) or Ventilator Associated Tracheobronchitis (VAT) during ICU stay up to a maximum of 90 days post randomisation as evaluated by an independent endpoint adjudicator, blinded to the randomisation arm and independent of patient care. For both VAP and VAT, it was a core requirement that the patient had been intubated for at least 48 hrs, and that the tube was in place within the 48 hours before the infection occurs. An additional core criterion for all cases was that a decision has been made to start new antibiotics or change the antibiotic regimen to treat a new infection. In addition to these core criteria, the endpoint diagnosis of VAT further required new onset of purulent respiratory secretions or change in character of sputum or increase in volume of sputum plus either (1) temperature >38 °C or < 36 °C or (2) white blood cell count <4.0 x 10^9/L or ≥12 x 10^9/L with no other recognised cause. The endpoint diagnosis of VAP was met when

the core criteria were met and there were new or progressive changes on chest radiography plus two of following three criteria (1) temperature >38 °C or < 36 °C, (2) white blood cell count <4.0 x 10^9/L or ≥12 x 10^9/L with no other recognised cause and (3) a new onset of purulent respiratory secretions or change in character of sputum or increase in volume of sputum.

Secondary endpoints included microbiologically confirmed VARI (defined as VARI plus bacterial growth of ≥10^5 colony forming units (CFU)/ml (endotracheal aspirate) or equivalent semi-quantitative growth), clinical and microbiologically confirmed VAP, proportion of intubated days receiving antibiotics, incidence of other HAI, including hospital acquired bloodstream infection (defined as either (1) least one positive blood culture for a recognised pathogen or (2) at least two positive blood cultures for a common skin contaminant (from two separate blood samples within 48 hours) and either fever (> 38 °C) or chills or hypotension (as defined by the European Centre for Disease Prevention and Control (ECDC)) (European surveillance of healthcare-associated infections in intensive care units, 2015) whilst intubated, total number of days ventilated/in ICU, cost of ICU/hospital stay, cost of antibiotics during ICU/hospital stay and mortality at 28 and 90 days after randomisation, and at ICU. Safety data including in-hospital re-intubation, tracheomalacia, tracheal stenosis and other local complications of ET placement were collected up to 90 days post randomisation, or up to hospital discharge, whichever was later. The time periods over which endpoints or censoring events were elicited is shown in Table 2-6.

2.4.5. Sample size

Based on local data and that from a large point-prevalence study in Vietnam (Phu et al., 2016) the estimated period prevalence of VARI (the primary outcome) in non-tetanus and tetanus ventilated patients is 20% and 30% over the course of their admission respectively. Based on previous small studies we expected an effect size of a 50% reduction in VARI using CPC, but wished to be able to detect a 40% reduction (Lorente et al., 2014, Nseir et al., 2011). This effect size was expected to be the same in both tetanus and non-tetanus patients. To preserve the generalisability of the study for settings where tetanus is less common, whilst still demonstrating the utility of the intervention in this patient group, we stratified randomisation to ensure that 30% of our recruited patients had a diagnosis of tetanus. Thus, we might expect to reduce VARI rates from 20% to 12% in non-tetanus and

from 30% to 18% in tetanus patients). In a study population with 30% tetanus patients, this corresponds to an absolute risk reduction from 23% to 13.8%. To detect this reduction with 80% power at the two-sided 5% significance level, 278 patients were required in each arm. Allowing for 8% loss to follow up we aimed to recruit 600 patients in total (420 non-tetanus, 180 tetanus).

Table 2-6. Follow up periods for all study endpoints.

Endpoint	Beginning of follow up	End of follow up/censoring event (soonest event applies except where specified)
Primary:		
VARI	Randomisation	ICU discharge/death/transfer or 90 days
Secondary:		
Microbiologically confirmed VARI	Randomisation	ICU discharge/death/transfer or 90 days
Clinical & Microbiologically confirmed VAP	Randomisation	ICU discharge/death/transfer or 90 days
Intubated days receiving antibiotics	Randomisation	ICU discharge/death/transfer or 90 days
Incidence of HAI	Randomisation	Extubation/death/transfer/discharge from ICU or 90 days
Days ventilated/in ICU	Randomisation	ICU discharge, death, transfer
Cost of ICU stay	ICU admission	ICU discharge/death/transfer
Cost of antibiotics in ICU stay	ICU admission	ICU discharge/death/transfer
Cost of hospital stay	Hospital admission	Hospital discharge
28-day mortality	Randomisation	28 days after randomisation
90-day mortality	Randomisation	90 days after randomisation
ICU mortality	Randomisation	Discharge from ICU or death/palliative discharge from it
Hospital mortality	Randomisation	Discharge from hospital or death/palliative discharge from it

2.4.6. Randomisation procedure

Randomisation was 1:1 stratified by site and whether or not the patient had a clinical diagnosis of tetanus at the time of randomisation. A stratified, computer-generated

randomisation list was created using block randomisation with variable block length and incorporated into secure internet accessible software that implements the randomisation. Once an eligible patient had consented, the initials and date of birth of the patient were entered into the software by study staff. Based on the randomisation list, the software produced the treatment allocation, which was displayed and recorded in the study database. All entries and outputs of the software were auditable.

2.4.7. Intervention

Depending on the results of randomisation patients received either manual, intermittent endotracheal cuff pressure control, which was checked and adjusted 8 hourly (standard care), or automatic, continuous endotracheal CPC (intervention group). Target pressure in both groups was 25 cm H_20 as a default. Changes to this target were recorded and the clinical reason for the change noted.

2.4.8. Study assessment

On a daily basis, the attending physicians assessed whether there was a new infection in a patient who had been intubated for ≥ 48 hours and in whom the tube was still in place or had been removed within the previous 48 hours. If the answer was 'yes', a standard battery of tests including complete blood count, procalcitonin, arterial blood gas, blood culture, sputum/endotracheal aspirate microscopy and culture, urine culture and chest x-ray was performed regardless of the suspected site of infection. Test results and clinical details from up to 5 days previous, the day of the HAI evaluation and subsequent 2 days were collected, including maximum/minimum temperature, changes in ventilation parameters (fractional inspired oxygen, positive end expiratory pressure), changes in sputum colour or volume, new inotrope or vasopressor requirements. The treating clinicians provided their own diagnosis of the aetiology of the infection according to ECDC criteria and prescribed antibiotic and other therapies as per routine care. However, for the primary endpoint, an endpoint reviewer, blinded to the treatment allocation and independent of clinical involvement with the patient, reviewed the complete case report form and radiology of patients completing the study at the end of each month to determine whether they met the primary or appropriate secondary endpoint criteria.

Enrolled patients had cuff pressure controlled in accordance with the allocated study arm for the entire duration of their intubated time on ICU. Follow up for the primary

outcome was until transfer to another facility, death, discharge from ICU or 90 days following randomisation, whichever was soonest. Patients that required re-intubation during the same stay in ICU continued in the study, using the same cuff pressure control measures originally allocated to them. Patients discharged from ICU but requiring return to ICU and re-intubation were not re-enrolled and were managed in accordance with standard care. At the end of the hospital stay the costs of the stay as billed were recorded (including bed, ventilator and other supportive therapies costs, drugs including antibiotic costs, but not including labour as per Vietnamese Ministry of Health guidance). Additionally, as a part of secondary endpoints assessment, study staff telephoned patients or their relative at 28 and 90 days after randomisation if they were no longer inpatients in order to assess mortality and complications related to intubation. Study procedures with a schedule of enrolment, interventions and assessments in Table 2-6.

Table 2-7. Schedule of enrolment, interventions, and assessments

	STUDY PERIOD								
	Enrolment	Allocation	Post-allocation						Close-out
TIMEPOINT**			*Daily basis*	*HAI suspicion*	*Extubation*	*ICU discharge*	*Hospital discharge*	*Day 29*	*Day 90*
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Randomisation		X							
INTERVENTIONS:									
Continuous endotracheal cuff pressure control		X	←	—	→				
Intermittent endotracheal cuff pressure control (q8h)		X	←	—	→				
ASSESSMENTS:									
Demographic data	X								
Charlson Comorbidity Index & APACHE II	X								
PEEP and FiO2		X	X	X					
HbA1c		X							
Chest x-ray		X		X					
Cuff pressure measurement		X	X						
Complete Blood Count		X		X					
Arterial blood gas analysis		X		X					
Procalcitonin				X					
Blood and urine culture				X					
Respiratory secretions microscopy & culture				X					
Risk factors for VARI *Use of sedative agents* *Use of paralytic agents* *Feeding* *Use of PPI*			X						
Mortality			X			X	X	X	X
Endotracheal tube cuff related complications			X			X	X	X	X
Cost of stay						X	X		
Cost of antibiotics						X	X		

2.4.9. Data management

Relevant data was recorded onto a case report form (CRF) and checked for accuracy. After the follow up was finished, the CRFs were deidentified by removing patient identifiers

and telephone numbers. Selected staff were trained on how to enter all clinical data from the CRFs and from laboratory source documents into a computerised data entry system using double data entry. Data will be retained for at least 15 years after completion of the study (last patient followed up for 90 days). Original paper documents will be maintained for at least 5 years, thereafter digital copies will be retained. All stored records are kept secure and confidential.

All personal information reviewed as a part of this study was remain confidential. Patient names or identifiers were not appeared in any database. Data cleaning was performed to detect and edit ambiguous or missing data. The database was locked and statistical analysis plan was agreed before final analysis and unblinding study arms.

2.4.10. Statistical Analysis

2.4.10.1. Data analysis for Chapter 6

Standard descriptive statistics were calculated for categorical (in percentage) and continuous variables (median and interquartile, IQR). Bivariate analyses were performed using Pearson's chi-squared test or Fisher's Exact Test for categorical variables as appropriate. Cox proportional-hazards regression was used to identify variables that predict the occurrence of HABSI and clinical outcomes. Cox's proportional hazards regression was performed to analyse factors associated with all-cause in-hospital mortality. All tests were two-tailed and differences were considered statistically significant at *p* values ≤0.05.

2.4.10.2. Data analysis for Chapter 7

Among patients who were enrolled for the clinical trial, I calculated standard descriptive statistics for categorical (in percentage) and continuous variables (median and interquartile, IQR) for baseline characteristics of all patients and the subgroup of patients with VARI and VAP. No statistical tests were performed to test statistical significance of characteristics among different groups. The statistical analysis plan is being finalised prior to unblinding of the trial and analysis of data.

2.4.11. Ethics approval and consent to participate

Patients who required intubation at the study site on or after arrival or who had been intubated prior to transfer, or their legal representatives, were approached as soon as possible in line with prioritising patient care, fully informed and invited to consent to the

study. Consent was obtained by study or hospital staff trained in good clinical practice (GCP) and the consent procedure. Randomisation then took place and patients were managed in the appropriate manner. Patient care was never delayed for consent or randomisation procedures. Where consent was provided by a patient's representative and they later regained the capacity to provide consent, the patient was approached and given the opportunity to withdraw from the study.

This study protocol and the template informed consent forms were reviewed and approved by the Ethical Committee in Biomedical Research - Hospital for Tropical Diseases (Approval number 30/QĐ-BVBNĐ), the Institutional Review Board – National Hospital for Tropical Diseases (Approval number 22/HDDD-NDTU), the Ethical Committee in Biomedical Research Trung Vuong Hospital (Approval number 670—BVTV) and the Oxford Tropical Research Ethics Committee (OXTREC Reference: 26-16) with respect to scientific content and compliance with applicable research and human subjects regulations. The trial was registered with Clinicaltrials.gov (NCT02966392).

2.4.12. Reporting of Adverse Events & Monitoring

As adverse events are relatively common in critically ill patients intubated on the ICU, safety reporting focused on events of potential relevance to cuff pressure control. The events being reported to the ethical committees were (1) all unexpected serious adverse events, (2) all serious adverse events judged to be related or possibly related to cuff pressure and (3) all deaths or palliative discharges (discharge home of a patient where ongoing care was considered futile to permit the patient to die at home, a commonly preferred alternative to dying in hospital in Vietnam). Reporting to the ethical committees was in accordance with GCP.

An independent Data and Safety Monitoring Board (DSMB) was established consisting of expert Vietnamese and international researchers and doctors, with the necessary clinical, research and statistical knowledge. The DSMB reviewed the protocol, a DSMB charter outlines its responsibilities and how it operates. All DSMB reports were sent to the responsible ethical committees including the site ethical committees, the Oxford Tropical Research Ethics Committee for consideration. Recruitment was continued at active sites during the DSMB review period. No interim analyses were planned for the primary endpoint, however, safety analyses was be conducted after 30 patients were recruited and subsequently at points decided by the DSMB.

The study was monitored by a team at OUCRU, independent of study design and management. Central monitoring took place regularly with on-site monitoring occurring prior to commencement of the study, after the recruitment of 25 patients in total, at 12 months and at intervals prior to and after 12 months depending on the results of early monitoring visits.

2.4.13. Funding

The study was funded by the Wellcome Trust Asia Programme grant (grant code 106680/Z/14/Z).

Chapter 3: **Price and use of antimicrobials in an emerging pharmaceuticals market in Vietnam**

3.1. Introduction

Despite concerted international efforts, antimicrobial use continues to rise in both humans and animals. Data on antibiotic sales from 76 countries (including Vietnam) between 2010 and 2015 estimated global antibiotic consumption in humans has increased by 65% over this period, reaching 42 billion defined daily doses (DDDs) every year (Klein et al., 2018). Global consumption is forecast to increase by a further 200% between 2015 and 2030 if there are no changes in current practice (Klein et al., 2018). Overall antibiotic consumption (in DDDs per 1 000 population per day) differs 3 fold between countries with an up to 16 fold variation in volumes of quinolones and cephalosporins among the mostly high-income member states of the Organisation for Economic Co-operation and Development (OECD) (OECD, 2017). Antibiotic consumption was positively correlated with growth in per capita gross domestic product (GDP) (Klein et al., 2018) and low- and middle-income countries (LMICs) are disproportionately responsible for driving the rise in global antibiotic consumption (Van Boeckel et al., 2014). From 2000 to 2010, Brazil, Russia, India, China and South Africa (BRICS) contributed 30% of global population growth but 76% of the increase in global antibiotic consumption (in number of doses) in the same period (Van Boeckel et al., 2014).

There is a positive correlation between antibacterial consumption and levels of bacterial resistance to antibiotics (Goossens et al., 2005). WHO introduced the AWaRe classification of antibacterials (Access, Watch and Reserve groups) to promote antimicrobial stewardship at local, national and global level, and address the challenge of increased antimicrobial resistance. The 'Access' group includes first and second choice antibacterials for the empirical treatment of common infectious syndromes which should be widely available in all healthcare settings. The 'Watch' group includes antibacterial classes that have higher resistance potential and are recommended for a specific, limited number of indications. Finally, the 'Reserve' or last resource group includes antibacterials that are recommended for highly specific patients when all alternatives have failed (see appendix 1 for detailed classification) (World Health Organization, 2017c). WHO

recommends that countries monitor the consumption of the Watch and Reserve antibacterials carefully as part of their AMR strategy (World Health Organization, 2017c) and to timely inform policies to optimize the use of antibacterials (World Health Assembly, 2015).

Access category antibacterials comprise more than 50% of total consumption (in DDD per 1000 inhabitants per day) in 49/65 reported countries in the period of 2015-2016 , whilst the Reserve category accounted for less than 2% of total antibacterial consumption in DDD/1000 person days in most high-income countries. In the European region, the Reserve category was rarely used, amounting to a median of 0.2% of all antibacterial use in 45 countries surveyed (World Health Organization, 2018d).

Vietnam is a lower middle-income country (LMIC) with a population of 94.6 million and GDP per capita of US$2,171 (World Health Organization, 2018c). In 2016, health expenditure accounted for 5.7% of GDP, corresponding to annual per capita health expenditure of US$122.8, 45% of which was out of pocket spending (World Health Organization, 2018c). However, data on antibiotic consumption from LMICs are still limited, especially for countries from South East Asia (World Health Organization, 2018d). In addition to quantifying consumption of antimicrobials, understanding relative costs and spending on antimicrobials is important since antimicrobial consumption is associated with antimicrobial resistance but a high price of medication may act as a barrier to access, reducing consumption. Our study aimed to estimate antimicrobial usage, expenditure and cost in public hospitals in Vietnam.

3.2. Materials and Methods

Data on antimicrobial procurement were obtained from tender-winning bids from 52/63 provincial health authorities and 30 public hospitals across Vietnam from 2018. Antimicrobials were classified using the Anatomical Therapeutic Chemical (ATC) Index and the WHO Access, Watch, Reserve (AWaRe) groups. Volume was measured in number of Defined Daily Doses (DDD). Antimicrobial cost were presented per DDD. The detail of method was presented in Chapter 2.

3.3. Results

We included tender-winning results totalling US$1.68 billion from 23 secondary hospitals, 7 primary hospitals and 52 provincial departments of health in Vietnam. With

estimation of total pharmaceutical sales in Vietnam in 2018 was US$ 5.85 billion (Business Monitor International Ltd., 2016), our analysis (US$1.68 billion) may represent 28.7% of the national budget for medication. The overall budget for systemic antibacterials and antifungals accounted for 28.6% (US$482.6 million) of the total drug budget for the study hospitals (Table 3-1).

Among antibacterials for systemic use (J01), there were a total of 77 different substances (ATC 5th level) in 23 chemical subgroups (ATC 4th level) procured over all sites. Antibacterials procured according to their AWaRe categories are presented in Figure 3-1. Overall, 25.8% of DDD (178.658.638 DDD) procured were unclassified by the AWaRe index, among which 78.5% were 2nd generation cephalosporins (J01DC), 13.4% were 1st generation cephalosporins (J01DB), 5.4% were macrolides (J01FA) and 2.7% were other substances. The Access group, Access and Watch group and Watch group antibacterials accounted for 42.9%, 23.4% and 7.7% of procured number of antimicrobial DDD respectively whilst the Reserve group accounted for 0.3% of antibacterials procured. The proportion of Access group, Watch group and Reserve group provided by domestic manufactures were 83.2%, 83.7% and 43.1% respectively.

Table 3-1. Budget and number of DDD

		Department of Health (n=52)	Secondary hospital (n=23)	Primary hospital (n=7)	All sites
J01_antibacterials for systemic use	Total budget (%)	US$430,713,755 (29.50%)	US$47,888,882 (22.01%)	US$1,426,924 (18.49%)	US$480,029,561 (28.48%)
	Number of DDD (%)	636,851,337 (93.26%)	51,468,583 (95.32%)	4,036,865 (96.62%)	692,356,785 (93.43%)
J02_antimycotics for systemic use	Total budget (%)	US$1,750,265 (0.12%)	US$815,819 (0.37%)	US$13,898 (0.18%)	US$2,579,982 (0.15%)
	Number of DDD (%)	2,342,056 (0.34%)	US$219,822 (0.41%)	20,798 (0.50%)	2,582,676 (0.35%)
P01_antiprotozoals	Total budget (%)	US$940,694 (0.06%)	US$17,942 (0.01%)	US$1,577 (0.02%)	US$960,214 (0.06%)
	Number of DDD (%)	9,657,984 (1.41%)	406,181 (0.75%)	34,875 (0.83%)	10,099,040 (1.36%)
P02_anthelmintics	Total budget (%)	US$436,790 (0.03%)	US$68,622 (0.03%)	US$2,369 (0.03%)	US$507,781 (0.03%)
	Number of DDD (%)	4,735,531 (0.69%)	590,841 (1.09%)	17,250 (0.41%)	5,343,622 (0.72%)
Other medications	Total budget (%)	US$1,026,229,647 (70.29%)	US$167,855,286 (77.16%)	US$6,225,722 (80.66%)	US$1,188,532,427 (70.52%)
Total	Total budget (%)	US$1,460,071,152 (100.00%)	US$217,553,353 (100.00%)	US$7,718,581 (100.00%)	US$1,685,343,086 (100.00%)
	Number of antimicrobial DDD (%)	682,893,636 (100.00%)	53,994,079 (100.00%)	4,178,096 (100.00%)	741,065,810 (100.00%)

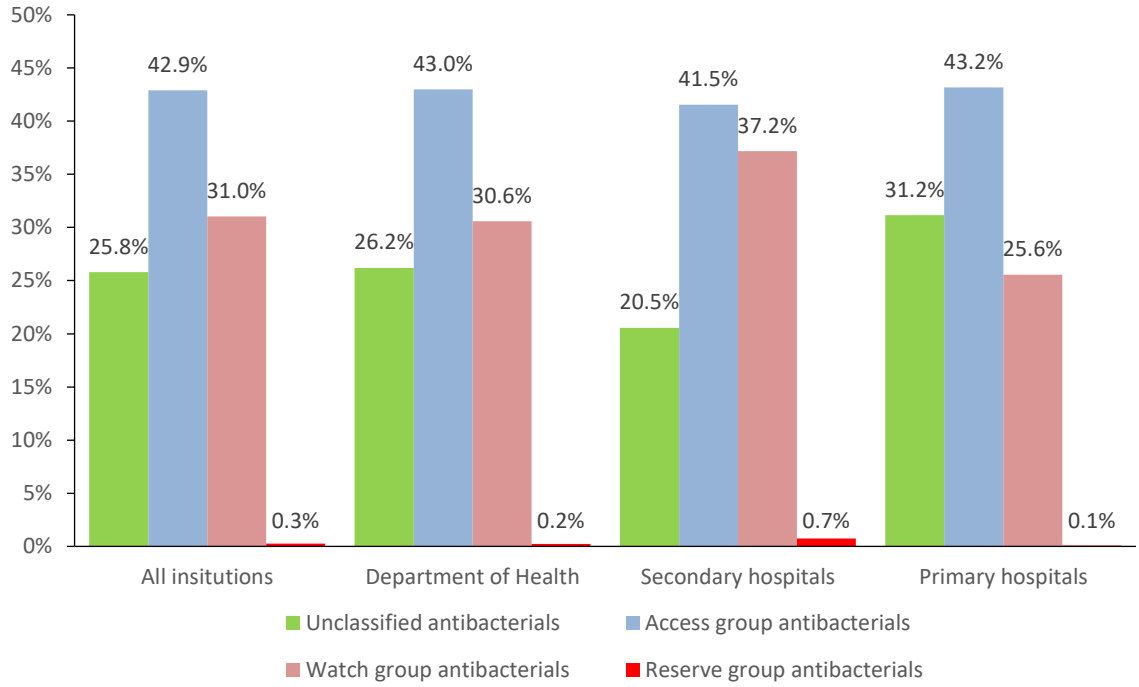
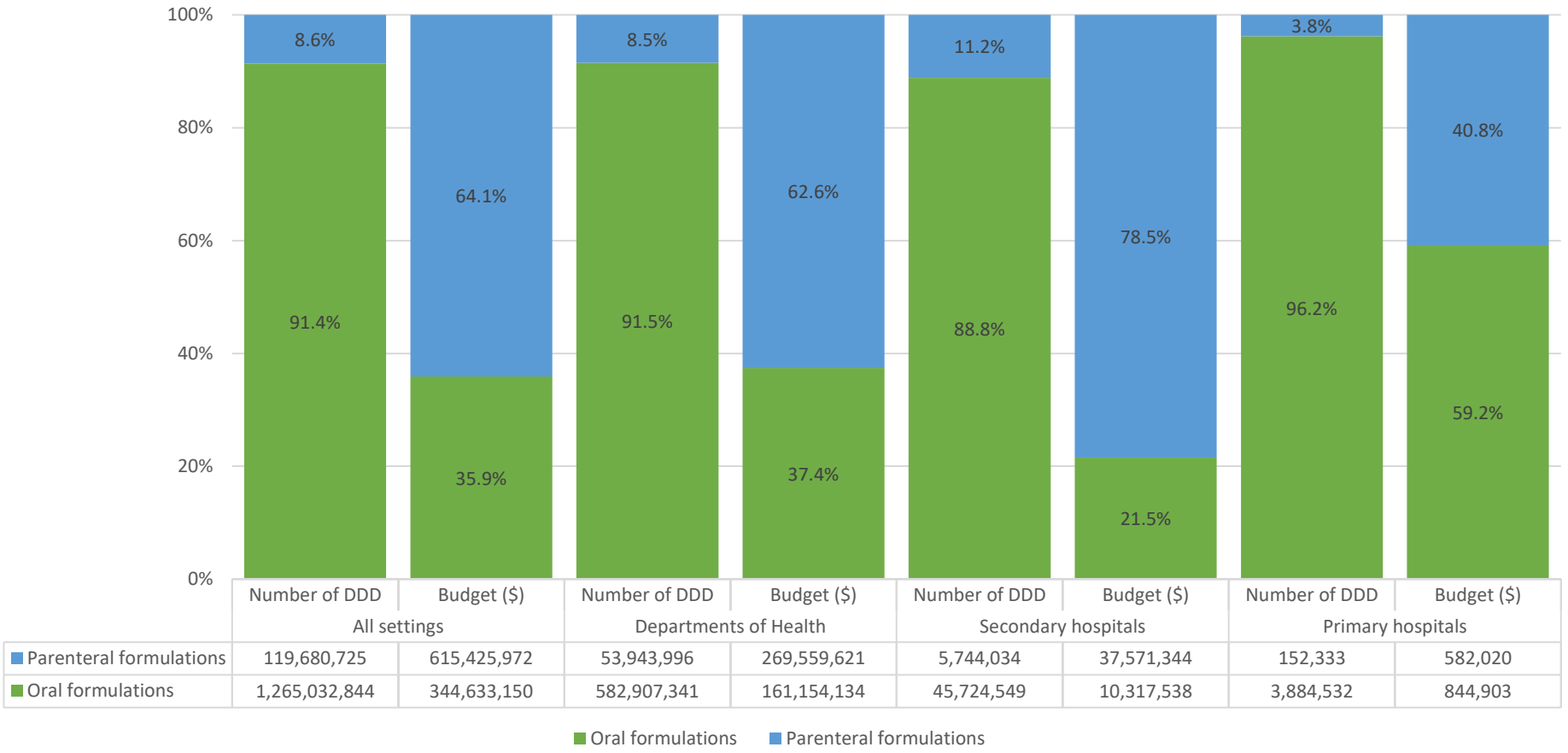


Figure 3-1. Proportional antibacterial procurement in DDD (%) by AWaRe classification

Oral antibacterials accounted for 91.4% of total DDD of antibacterials (J01) across all sites (Figure 3-2). Parenteral antibacterials represented 11.2% of the procured antibacterial DDDs in secondary hospitals, 3.8% in primary hospitals and 8.5% in bids by DoH. The most common oral antibacterial across all sites were the second generation cephalosporins (J01DC) (19.8% of total DDD). When stratifying by the site of procurement, the most common consumed oral antibacterials in secondary hospitals were combinations of penicillins and beta-lactamase inhibitors (J01CR) (29%) and in primary and departments of health hospitals were the second generation cephalosporins (J01DC) (21.8% and 20.3% respectively). For parenteral antibacterials, the most common antibacterials were the third generation cephalosporins (J01DD) (29.1%). The details of antibacterial procurement in DDD is shown in Table 3-2.

The cost shares of antibacterials procurement are shown in Table 3-2. The second generation cephalosporins (J01DC), combinations of penicillins, including beta lactamase inhibitors (J01CR), penicillins with extended spectrum (J01CA), third generation cephalosporins (J01DD) and fluoroquinolones (J01MA) covered 76.6% of the total quantity of DDD, reaching 65.7% of the total costs (Table 3-2). However, carbapenems (J01DH) only accounted for 0.3% of antibacterial use but 10.2% of the total antibacterial costs.



	Number of DDD	Budget ($)	Number of DDD	Budget ($)	Number of DDD	Budget ($)	Number of DDD	Budget ($)
	All settings		Departments of Health		Secondary hospitals		Primary hospitals	
Parenteral formulations	119,680,725	615,425,972	53,943,996	269,559,621	5,744,034	37,571,344	152,333	582,020
Oral formulations	1,265,032,844	344,633,150	582,907,341	161,154,134	45,724,549	10,317,538	3,884,532	844,903

Figure 3-2. The cost shares and number of DDD of antimicrobials for systemic antibacterial (J01) by route of administration in hospitals in Vietnam.

Table 3-2. The proportion of DDD number and budget shares of the antibacterials for systemic (J01).

	Department of Health		Secondary hospitals		Primary hospitals		All sites	
	% DDD	% budget	% DDD	% budget	% DDD	% budget	% DDD	% budget
J01DC_Second generation cephalosporins	20.29%	16.52%	19.66%	8.06%	21.81%	15.87%	20.25%	15.67%
J01CR_Combinations of penicillins, incl. beta lactamase inhibitors	16.31%	15.77%	26.33%	15.22%	18.35%	24.10%	17.06%	15.74%
J01CA_Penicillins with extended spectrum	15.78%	2.94%	10.67%	0.91%	11.89%	2.21%	15.38%	2.74%
J01DD_Third generation cephalosporins	12.23%	23.02%	19.73%	23.31%	8.55%	24.86%	12.77%	23.05%
J01MA_Fluoroquinolones	11.33%	11.56%	12.89%	14.89%	11.54%	7.94%	11.45%	11.89%
J01DB_First generation cephalosporins	9.00%	8.16%	0.80%	0.57%	15.00%	8.52%	8.43%	7.40%
J01FA_Macrolides	8.22%	3.41%	4.05%	1.31%	9.20%	8.81%	7.91%	3.22%
J01AA_Tetracyclines	1.79%	0.07%	1.78%	0.47%	2.13%	0.08%	1.79%	0.11%
J01CE_Beta lactamase sensitive penicillins	1.59%	0.08%	0.07%	0.00%	0.39%	0.03%	1.47%	0.07%
J01EA_Trimethoprim and derivatives	0.97%	0.18%	0.22%	0.01%	0.11%	0.01%	0.91%	0.16%
J01GB_Other aminoglycosides	0.86%	1.63%	1.45%	1.27%	0.72%	0.84%	0.90%	1.59%
J01XD_Imidazole derivatives	0.40%	1.29%	0.48%	0.81%	0.05%	0.22%	0.40%	1.24%
J01CF_Beta lactamase resistant penicillins	0.39%	0.90%	0.18%	0.29%	0.05%	0.13%	0.37%	0.83%
J01FF_Lincosamides	0.22%	0.74%	0.22%	0.93%	0.00%	0.00%	0.22%	0.76%
J01DH_Carbapenems	0.18%	9.18%	0.50%	18.74%	0.04%	1.27%	0.20%	10.12%
J01DE_Fourth generation cephalosporins	0.15%	2.25%	0.29%	3.43%	0.05%	2.04%	0.16%	2.37%
J01MB_Other quinolones	0.14%	0.05%	0.01%	0.00%	0.04%	0.04%	0.13%	0.05%
J01XX_Other antibacterials	0.05%	0.69%	0.37%	3.09%	0.04%	0.76%	0.08%	0.93%
J01XA_Glycopeptide antibacterials	0.05%	0.69%	0.23%	2.78%	0.03%	1.05%	0.06%	0.90%
J01BA_Amphenicols	0.03%	0.03%	0.00%	0.00%	0.01%	0.01%	0.03%	0.02%
J01XB_Polymyxins	0.01%	0.84%	0.07%	3.90%	0.01%	1.19%	0.02%	1.15%

The cells were colorized with red-yellow-green color scale by column. The highest values in a column were red, the average values were yellow, and the lowest values were green. DDD= Defined Daily Dose.

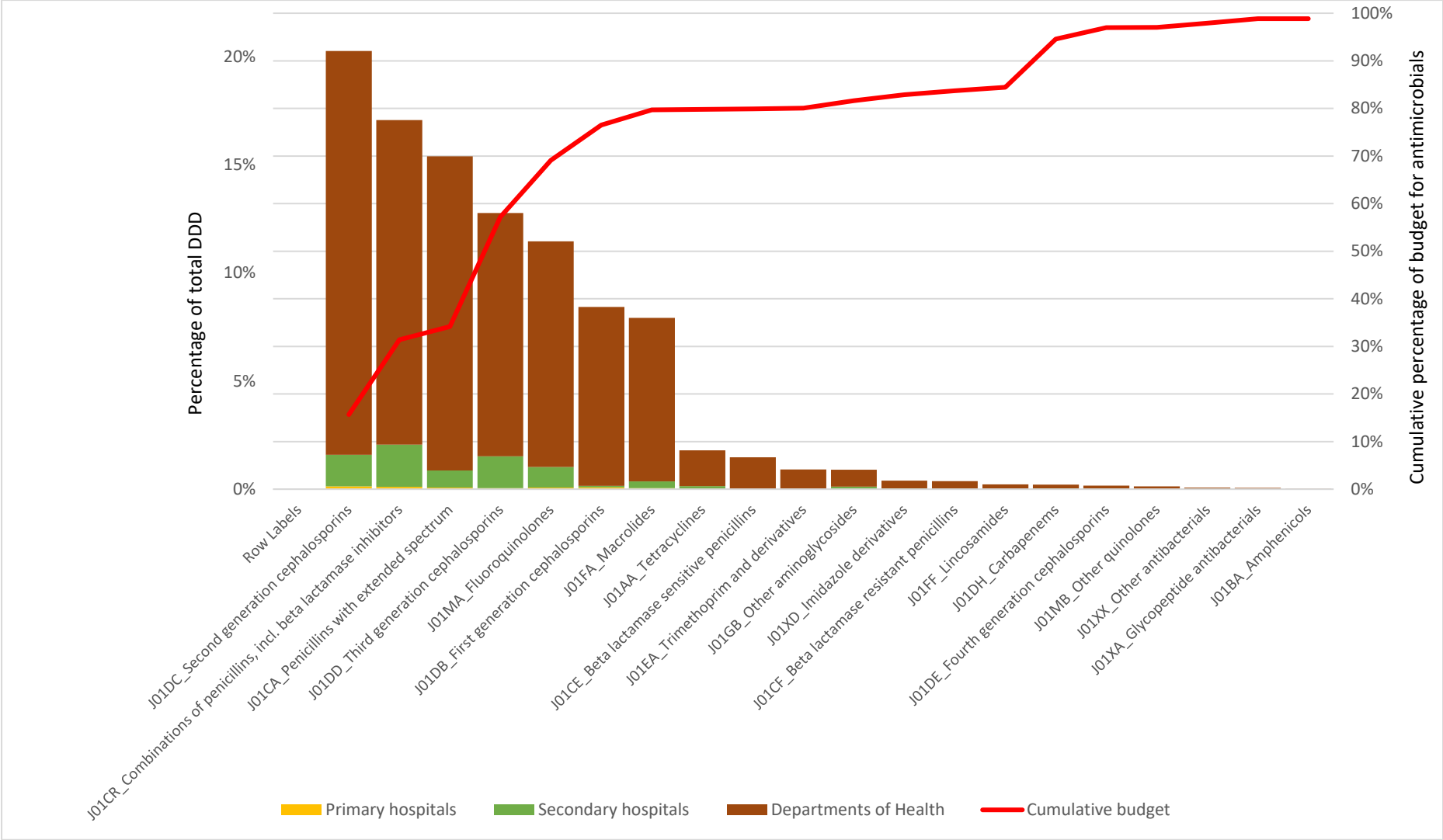


Figure 3-3. ABC analysis of the quantities of procured antibacterials (in DDD) and the cost.

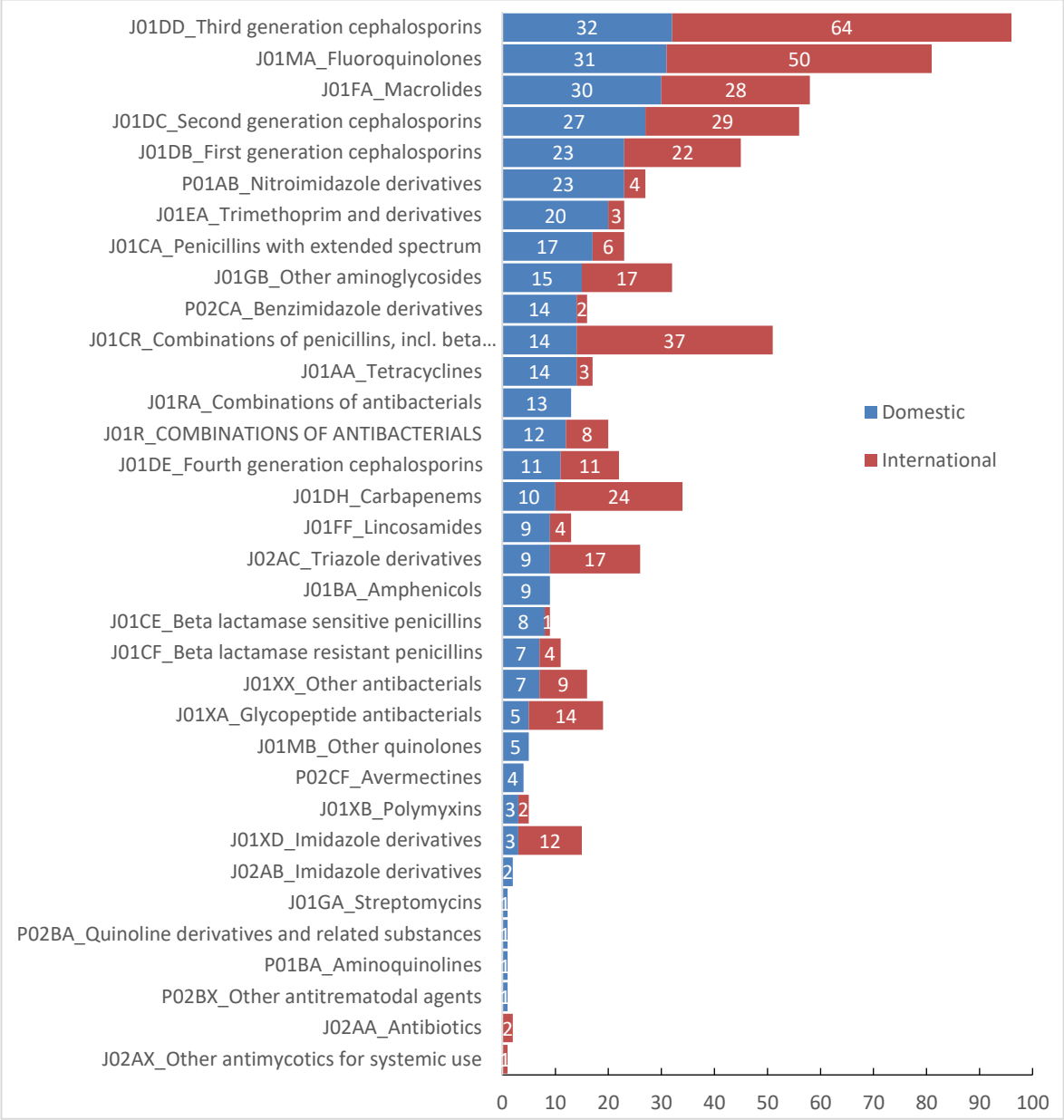


Figure 3-4. Number of antimicrobial manufacturers in Vietnam

By AWaRe categories, the average cost per DDD of Reserve group antibacterials was the highest (US$12.38 per DDD), followed by the Watch group antibacterials (US$2.15 per DDD), antibacterials in both Watch and Access group (US$0.74 per DDD), as unclassified antibacterials (US$0.64 per DDD) and Access group antibacterial (US$0.36 per DDD). We presented the cost of antimicrobials (ATC 5th level) in Table 3-4. The top three of the most expensive antimicrobials were caspofungin (J02AX04) (US$284.5 per DDD), doripenem (J01DH04) (US$85.3 per DDD) and tigecycline (J01AA12) (US$65.4 per DDD). There is a large variability of antimicrobial price per DDD range which is represented as a ratio of the highest to lowest price of antimicrobial per DDD (H/L ratio) from very high (H/L ratio up to 82 for oral formulation or 40.26 for parenteral formulation) or no discrepancy (H/L=0) for

the branded forms (Table 3-4). Twenty seven of thirty-eight oral forms (71.3%) and 10/55 parenteral forms (18.2%) of antimicrobials had H/L ratio above 10.

Whilst almost all (59/77 or 76.6%) antibacterials for systemic use (ATC 5th level, chemical substance) were dispensed from both domestic and international manufacturers, 18 were procured from either international or domestic manufactures (Figure 3-4 and Table 3-3). 82.7% of antibacterials were supplied by domestic producers (67 companies, supplied 572,698,014 DDDs) whilst 212 international manufactures from 35 countries supplied the remainder (119,658,771 DDD). Antibacterials supplied by international manufacturers accounted for 54.5% of the total costs (US$261,754,116) and 17.3% of the total DDD procured. Among 35 countries with pharmaceutical companies sharing the antibacterial market in Vietnam, India and Cyprus contributed the highest proportions of total DDD, together supplying 8.4% of total antibacterial (58,142,107 DDDs) in all sites, corresponding to 48.6% of total foreign antibacterials (J01) procured (28.6% by India and 20.1% by Cyprus).

Table 3-3. Source of manufacturers for selected antibacterials

Antibiotic procured by only domestics manufactures	norfloxacin and tinidazole (J01RA13) lomefloxacin (J01MA07) streptomycin (J01GA01) lincomycin (J01FF02) ciprofloxacin and tinidazole (J01RA11) ceftibuten (J01DD14) tetracycline (J01AA07) pefloxacin (J01MA03) benzylpenicillin (J01CE01) chloramphenicol (J01BA01) nalidixic acid (J01MB02) ticarcillin and beta lactamase inhibitor (J01CR03) oxacillin (J01CF04) gentamicin (J01GB03) spiramycin and metronidazole (J01RA04)
Antibiotic procured by only international manufactures	tigecycline (J01AA12) ertapenem (J01DH03) parenteral azithromycin (J01FA10)

Table 3-4. Prices of antimicrobials

Antimicrobials	All			Oral formulation			Parenteral formulation		
	No. Mfr.	Average price per DDD	High/low ratio	No. Mfr.	Average price per DDD	High/low ratio	No. Mfr.	Average price per DDD	High/low ratio
J02AX04_caspofungin	1	284.53	1.10	-			1	284.53	1.10
J01DH04_doripenem	4	85.31	1.56	-			4	85.31	1.56
J01AA12_tigecycline	1	65.36	1.00	-			1	65.36	1.00
J01XB01_colistin	5	49.98	2.18	-			5	49.98	2.18
J01CR03_ticarcillin and beta lactamase inhibitor	2	40.87	3.56	-			2	40.87	3.56
J01DH51_imipenem and cilastatin	21	35.71	6.33	-			21	35.71	6.33
J01CA12_piperacillin	2	34.88	1.80	-			2	34.88	1.80
J01DH02_meropenem	21	30.98	12.90	-			21	30.98	12.90
J01DC01_cefoxitin	13	26.57	8.32	-			13	26.57	8.32
J01DH03_ertapenem	1	24.69	1.00	-			1	24.69	1.00
J01CR05_piperacillin and beta lactamase inhibitor	14	18.51	3.90	-			14	18.51	3.90
J01DE02_cefpirome	9	18.00	3.59	-			9	18.00	3.59
J01XA02_teicoplanin	5	17.75	2.23	-			5	17.75	2.23
J01DD62_cefoperazone and beta lactamase inhibitor	21	16.56	27.33	-			21	16.56	27.33
J01DC03_cefamandole	8	15.88	4.35	-			8	15.88	4.35
J01DB03_cefalotin	4	14.55	1.77	-			4	14.55	1.77
J01CR01p_ampicillin and beta lactamase inhibitor	12	11.55	6.06	-			12	11.55	6.06
J02AA01_amphotericin B	2	11.30	12.10	-			2	11.30	12.10
J01DC09_cefmetazole	8	10.06	3.06	-			8	10.06	3.06
J01XX01_fosfomycin	8	9.12	2.81	2	5.13	1.52	6	10.31	2.62
J01DD07_ceftizoxime	15	7.95	6.18	-			15	7.95	6.18
J01DD12_cefoperazone	15	7.83	12.68	-			15	7.83	12.68
J01FA10p_azithromycin	6	7.62	6.90	-			6	7.62	6.90

Antimicrobials	All			Oral formulation			Parenteral formulation		
	No. Mfr.	Average price per DDD	High/low ratio	No. Mfr.	Average price per DDD	High/low ratio	No. Mfr.	Average price per DDD	High/low ratio
J01XA01_vancomycin	15	7.57	2.57	-			15	7.57	2.57
J01DE01_cefepime	16	7.18	19.53	-			16	7.18	19.53
J01MA14_moxifloxacin	16	6.93	37.69	7	0.92	5.38	9	12.09	3.06
J01CR02p_amoxicillin and beta lactamase inhibitor	12	6.58	6.52	-			12	6.58	6.52
J01DB12_ceftezole	10	6.07	2.90	-			10	6.07	2.90
J01DD02_ceftazidime	26	5.07	11.56	-			26	5.07	11.56
J01GB07_netilmicin	9	4.99	3.43	-			9	4.99	3.43
J01XX08_linezolid	8	4.67	40.45	4	1.70	1.57	4	24.76	3.68
J01XD02_tinidazole	4	4.61	2.98	-			4	4.61	2.98
J01DD04_ceftriaxone	28	3.51	40.26	-			28	3.51	40.26
J01DC07_cefotiam	10	3.05	4.00	-			10	3.05	4.00
J01DD01_cefotaxime	29	2.77	12.18	-			29	2.77	12.18
J01DB04_cefazolin	9	2.46	4.18	-			9	2.46	4.18
J01FF01_clindamycin	13	2.40	39.58	10	0.43	9.11	3	5.26	3.93
J01DD14_ceftibuten	2	2.37	2.46	2	2.37	2.46	-		
J01GB06_amikacin	12	2.25	8.33	-			12	2.25	8.33
J01CF02_cloxacillin	9	2.05	34.20	3	0.95	5.18	6	4.76	4.94
J01XD01_metronidazole	13	1.93	4.52	-			13	1.93	4.52
J01GB01_tobramycin	16	1.26	8.75	-			16	1.26	8.75
J02AC02_itraconazole	11	1.12	308.00	10	0.71	6.33	1	82.61	1.00
J01CF04_oxacillin	6	0.85	11.50	3	0.59	2.50	3	1.67	3.14
J01MA12_levofloxacin	39	0.81	495.43	20	0.27	72.13	19	5.07	12.04
J01DD13_cefpodoxime	27	0.76	27.03	27	0.76	27.03	-		
J01DC04_cefaclor	21	0.64	65.52	21	0.64	65.52	-		
J01DD15_cefdinir	19	0.64	29.79	19	0.64	29.79	-		
J01CA01_ampicillin	6	0.63	7.10	1	0.12	1.00	5	0.63	2.88
J01MA06_norfloxacin	6	0.61	18.73	6	0.61	18.73	-		
J01CE01_benzylpenicillin	4	0.61	5.40	-			4	0.61	5.40

Antimicrobials	All			Oral formulation			Parenteral formulation		
	No. Mfr.	Average price per DDD	High/low ratio	No. Mfr.	Average price per DDD	High/low ratio	No. Mfr.	Average price per DDD	High/low ratio
J02AC01_fluconazole	18	0.60	142.46	16	0.46	80.30	2	12.02	1.47
J01MA03_pefloxacin	5	0.58	12.50	3	0.14	1.71	2	1.10	1.27
J01MA02_ciprofloxacin	39	0.56	1899.69	24	0.08	42.81	15	8.85	31.10
J01BA01_chloramphenicol	9	0.52	5.25	8	0.46	2.02	1	1.34	1.14
J01FA02_spiramycin	17	0.45	9.07	17	0.45	9.07	-		
J01DB09_cefradine	8	0.43	12.76	5	0.42	12.76	3	0.74	3.62
J01CR02o_amoxicillin and beta lactamase inhibitor	33	0.38	23.70	33	0.38	23.70	-		
J01DB05_cefadroxil	24	0.37	24.89	24	0.37	24.89	-		
J01DD08_cefixime	31	0.34	52.05	31	0.34	52.05	-		
J01DB01_cefalexin	20	0.31	16.47	20	0.31	16.47	-		
J01MA01_ofloxacin	18	0.27	677.97	14	0.04	10.17	4	8.42	6.53
J01FA09_clarithromycin	23	0.27	19.19	23	0.27	19.19	-		
J01FA10o_azithromycin	27	0.26	38.66	27	0.26	38.66	-		
J01DC02_cefuroxime	39	0.25	28.92	28	0.21	28.92	11	0.58	9.54
J01MB02_nalidixic acid	5	0.25	1.83	5	0.25	1.83	-		
J01FA01_erythromycin	10	0.20	82.00	10	0.20	82.00	-		
J01FF02_lincomycin	1	0.17	1.67	1	0.16	1.00	-	0.27	1.00
J01GA01_streptomycin	1	0.16	1.00	-			1	0.16	1.00
J01GB03_gentamicin	9	0.15	2.24	-			9	0.15	2.24
J01EA01_trimethoprim	23	0.12	70.00	23	0.12	70.00	-		
J01FA06_roxithromycin	14	0.11	17.14	14	0.11	17.14	-		
J01CA04_amoxicillin	21	0.11	26.52	21	0.11	26.52	-		
J01AA07_tetracycline	9	0.04	1.76	9	0.04	1.76	-		
J02AB02_ketoconazole	2	0.03	1.19	2	0.03	1.19	-		
J01CE10_benzathine phenoxymethylpenicillin	7	0.03	2.74	7	0.03	2.74	-		
J01AA02_doxycycline	11	0.01	6.25	11	0.01	6.25	-		

3.4. Discussion

Our study represents the first effort to describe the use and cost of antimicrobials in healthcare facilities in Vietnam, a country with a high burden of drug resistant infections. In a previous study of antibiotic sales in 76 countries between 2000 and 2015, Vietnam ranked 11th in antibiotic consumption per capita with 32 DDDs per 1,000 inhabitants per day (Klein et al., 2018). However, the use of antimicrobials by ATC index and by the route of administration in public hospitals has not been published. Additionally, we found that antibacterials proportion of total medication expenditure was high - comprising 28.5% of the total budget for medications in all study sites.

We found that a quarter of antimicrobials (in DDD) were unclassified by AWaRe classification system. Similarly, a large proportion of unclassified antibacterials was also reported in other countries, for example 60.3% in Finland in a survey of prescriptions among hospitalised children in 56 countries in 2015 (Hsia et al., 2019). Thus, the current AWaRe system, fails to classify many of the most commonly used drugs. This limitations of AWaRe classification system was acknowledged the WHO Essential Medicines List Working Group and may require further revision (Sharland et al., 2018).

By classes of antibacterials, we found that cephalosporins were the most commonly prescribed in Vietnam. In healthcare sectors in Europe, where the resistance levels are low, the most common antibacterials were beta-lactams, penicillins (J01C) (European Centre for Disease Prevention and Control, 2018) whilst in China, where the resistant levels are similar to Vietnam, 3rd-generation cephalosporins were the most consumed antibiotic in hospitals (Yin et al., 2017). In the first surveillance report by WHO on antibiotic consumption in 65 countries during the period of 2016-2018, the Philippines was the only country from Southeast Asia providing data on national consumption of antimicrobials using IQVIA and import database (World Health Organization, 2018d). In Philippines, the most frequently consumed antibiotics were tetracyclines and penicillins with each contributing 30% of total consumption of antibiotics (in DDD per 1000 inhabitants per day).

My study provides important data concerning costs of antimicrobials used in Vietnam. The majority of antibiotic costs related to the cost of the most commonly used antibiotics according to DDD. However, a small number of antimicrobials accounted for a disproportionately large high expenditure. Carbapenems were only a small number of antimicrobials prescribed according to DDD, but a significant proportion of costs. Data on

the national expenditure on antimicrobials is often limited and there are few data from other countries for comparison but it is likely that the situation of last resort antibiotics is similar in other settings. For example, in the US in 2015, the antibiotic expenditures were largest for daptomycin (1.3 billion or 14.7% of total expenditure), and although no data are provided on daptomycin usage, it is likely that this is a relatively infrequently used antibiotic.

With a total of 290 manufacturers sharing the antimicrobial market in Vietnam, currently, there are numerous manufacturers with different forms and formulation of antimicrobials on the Vietnamese market. For example, there were eight US Food and Drug Administration–approved manufacturers for Cefuroxime 250 mg tablet in US (Alpern et al., 2017) whilst there were 20 manufacturers approved by Vietnam government for the same formulation and strength in Vietnam. This large number of domestic and international manufacturers likely leads to increased complexity and cost in ensuring the quality of medications and regulating their manufacture and distribution.

My study has shown high variability in cost of antimicrobials. Measurement of price and availability of antimicrobials is essential to inform policies about accessibility and affordability to the population. In a survey of the prices, availability, and affordability of 42 core medicines (including eight antibiotic substances) in 5 provinces in Vietnam in 2005, the prices of innovator drugs and the lowest priced generics were 47 times and 11 times higher than the international reference prices (MSH), respectively (Nguyen et al., 2009). The medicine prices in the public sectors were higher than in private sectors (Nguyen et al., 2009). The International Medical Products Price Guide by Management Sciences for Health (MSH) is recommended as a most useful reference for medicine prices (World Health Organization, 2008) and the 2015 version is the latest (Management Sciences for Health (MSH), 2015). Among antibacterials in the Reserve group, the high/low ratio of cefepime according to MSH 2015 (4.39) was much lower than the one in my study (19.53) (Management Sciences for Health (MSH), 2015). However, no data for other antibacterials in the Reserve group were available for cross reference.

The pharmaceutical market in Vietnam is import-reliant and was estimated to reach US$5.2 billion in 2017 (Business Monitor International Ltd., 2016). Ninety percent of the country's medication expenditure was on imported medicines (Angelino et al., 2017). The European Union was the most important pharmaceutical manufacturer providing

medications to Vietnam with a value of US$1.1 billion or 51 % of Vietnam's total pharmaceutical imports in 2014, in which France, Germany, Italy shares US$579 million or 73 % of total pharmaceutical imports from the EU (Vu, 2016). We confirmed that Europe were the leading exporters to Vietnam in term of values of antibiotics expenses whilst Asian countries (mainly India) exported the largest quantities. However, the majority of antimicrobial consumption was met by domestic manufacturers, which was part of strategic plan for developing the Vietnam pharmaceutical industry by 2020 as the government set objectives to produce 80% of total annual medication consumption in the country (The Prime Minister, 2014).

Our study has some limitations. Firstly, I was unable to obtain data from all healthcare institutions in Vietnam due to bids being published elsewhere, e.g. an institutional website. Based on the previous estimation of national drug expenditure (US$ 5.85 billion), we estimated our data represents at least 28.7% but this may be an underestimate because the total pharmaceutical sales of 5.85 billion included community pharmacies. Furthermore, of the data that were available, of antibacterial use from all sites were not complete because hospitals may have extra calls for bids or not use all of the antibiotics purchased from these bids. However, the successful bids were based on the previous year's actual (total) consumption and the institutions were required to use at least 80% of antimicrobials purchased in these bids, therefore our estimate, whilst not exact, is likely to be a reasonable estimate of use. Our estimates of antimicrobial usage in hospitals may be biased and underestimate exact usage because medications for hospitalised patients can also be purchased directly by patients, especially in primary and secondary hospitals because many Watch and Reserve antibacterials are not reimbursed by the national insurance programme and consequently not available in hospital pharmacies (Viet Nam Ministry of Health, 2018). Additionally, there is some uncertainty about the number of hospitals included in this study as the bid by DoH covered the tenders of different public hospitals in the province and the number of hospitals joining the provincial bidding was unidentifiable.

In future with the advent of an electronic health record (currently being implemented in Vietnam), it may be possible to obtain more accurate data and cross reference purchased drugs with prescribed drugs. Finally, this study did not include private sector consumption. As the Vietnamese Government launched a strategy to increase the share of private

hospital beds to 20% by 2020 (Vietnam's Prime Minister, 2013), the growth of the private healthcare system may contribute significantly to consumption of antimicrobials.

3.5. Conclusions

Antimicrobials accounted for a third of budget for medication in public hospitals in Vietnam. The pattern of antibacterial consumption by AWaRe categories was similar to other countries. The high cost of Reserve group antibacterials suggests a need for national pricing policies to ensure accessibility and the importance of implementation of antimicrobial stewardship. This high cost, however, may be seen as a barrier to inappropriate use of these antibiotics and may contribute to antibiotic stewardship.

Chapter 4: **Excess direct hospital cost of treating ventilator associated respiratory infection (VARI) in Vietnam**

4.1. Introduction

In recognition of the complexity of antimicrobial costs, in this chapter I provide some understanding on the cost of management of patients with ventilator associated respiratory infection (VARI) in Vietnam.

Hospital acquired infections (HAIs) are a major concern in healthcare institutions world-wide. HAI prevalence ranges from 4% in the United States (Magill et al., 2014) to 7.1% in Europe (Zarb et al., 2012). In a literature review of 6 countries from 41 studies in Southeast Asia between 2000 and 2012, HAI occurred in 20 patients per 1000 intensive care unit-days with the highest incidence density attributed to ventilator-associated pneumonia (14.7 per 1000 ventilator-days) (Ling et al., 2015). In critical care units (CCUs), approximately 30% of patients acquire HAI during their stay: (Vincent, 2003) the most common being ventilator-associated pneumonia (VAP) and ventilator-associated tracheobronchitis (VAT) . VAP and VAT can be grouped together as Ventilator Associated Respiratory Infections (VARI), a term that then encompasses the spectrum of respiratory infections that clinicians in Vietnam and many parts of the world treat with antibiotics (Craven et al., 2011). VARI is associated with excess length of stay, prolonged duration of ventilation, increased cost and, (in some studies) increased mortality (Melsen et al., 2013, Arabi et al., 2008). Incidence densities of 3.2-56.9 per 1000 ventilator-days have been reported in resource constrained setting (Allegranzi et al., 2011).

The direct financial costs of such infections have been reported to be high (Stone et al., 2005, Zimlichman et al., 2013), and it is important to improve our understanding of these costs in order to mobilise resources for prevention and control measures. In a systematic review of publications between 2001 and 2004, mostly in high-income settings, the mean excess cost of VAP was US$ 9,969 ± 2,920 (Stone et al., 2005). A more recent estimate from the USA found that the average excess cost per VAP case for an adult inpatient was $40,144 (36,286-44,220) (Zimlichman et al., 2013). In lower and middle income countries, the case-based cost is considered significantly lower but not well described (Mathai et al., 2015, Ling et al., 2015). However the incidence is higher (Allegranzi et al., 2011) and a greater proportion of the costs may be met out of pocket, leading to substantial impacts on the

patients and their families (Global Burden of Disease Health Financing Collaborator Network, 2017).

Vietnam is a lower-middle income country with a population of 93 million and a gross national income (GNI) per capita of US$ 2,060 in 2016 (World Bank, 2018a) . As of July 2016 Vietnam had 13,571 healthcare facilities, including 1,132 general and specialized hospitals with 305,700 in-patient beds (General Statistics Office, 2017, Vietnam Ministry of Health, 2016). This figure includes 75 central hospitals (including 38 national hospitals) that receive referrals from outside of the local province, 502 provincial or equivalent hospitals that receive referrals from within the local province, 548 districts hospitals and 7 unclassified hospitals that receive patients only directly from the community (Vietnam Ministry of Health, 2016).

Although in Vietnam, VARI is an increasing concern, little is known about its economic impact. High levels of antibiotic resistance (Phu et al., 2016) and infection control programmes which are often inadequate means this is increasingly important, and VARI has been shown to be a particular problem (Duong and McLaws, 2017, Phu et al., 2017).

The aim of this study was to estimate the total annual excess cost associated with VARI in critical care units in Vietnam. Such an estimate will help policy makers in planning and implementing necessary changes in infection control, antibiotic stewardship and other relevant policies. To address this, a costing model was constructed to evaluate the excess cost of diagnostics and treatment of VARI in Vietnam from a healthcare sector perspective using the incidence-based cost-of-illness approach

4.2. Materials and methods

I constructed a costing model to evaluate the excess cost of diagnostics and treatment of VARI in Vietnam. Model inputs included costs for extra lengths of stay, diagnostics, VARI incidence, utilisation of ventilators and antibiotic therapy. Cost for healthcare services were obtained from the national ceiling price of medical services in published hospitals by Ministry of Health and cost for antimicrobial was calculated from the 2017 medication bid-winning results of national and provincial hospitals in Vietnam. A sensitivity analysis was used to examine how the different cost of treatment components affect cost of VARI management under the current incidence rate of VARI. The detail of method was presented in Chapter 2.

4.3. Results

The average excess cost of VARI management in national and provincial hospitals in Vietnam was US$ 1,174.90 per episode (range from US$ 682.10 - 1,667.60) with the cost of antibiotic treatment comprising US$ 600.20 (US$ 247.60 - 952.90) or 51.1% of the total cost of VARI episode. The share of average cost components per VARI episode were shown in Figure 4-1.

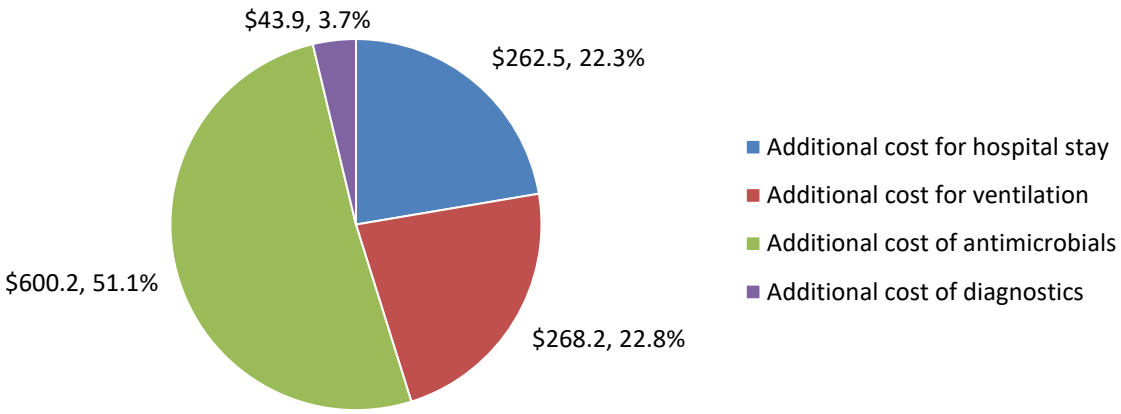


Figure 4-1. The share of cost components per VARI episode.

Based on assumptions presented in Table 2-2, the estimated number of cases of VARI in Vietnam was 34,428 episodes annually, at a VARI incidence of 21.7/1,000 ventilation days (Figure 4-2). The estimated excess cost of VARI nationwide was US$ 40,447,469 (range US$ 23,482,151 - 57,412,787). An absolute reduction in VARI incidence density of 1% led to a decrease of 1,578 episodes per year and a saving of US$ 1.86 million nationally.

The cost of 12 days antimicrobial therapy for VARI contributed US$ 20,664,650 (US$ 8,524,541-32,804,759) to the national cost and a decrease in cost of US$ 1.72 million per one day reduction in the duration of antimicrobial therapy (assuming no impact on outcome). The average daily cost of antimicrobial to treat carbapenemase-producing Gram-negative bacteria was US$ 172 (US$ 110-235.3) versus US$ 28.3 (US$ 7.7-48.9) to treat carbapenem susceptible Gram-negative bacteria. Based on current data on the aetiology and resistance patterns of bacteria causing VARI (Phu et al., 2016), the proportion of VARI caused by carbapenemase-producing Enterobacteriaceae (CPE) organisms was 16.4% (corresponding to 5,633 VARI episodes per year). However, the costs of antimicrobial treatment alone of these episodes amounted to US$ 11,733,107, accounting for to 29% of the total national cost of VARI management.

Figure 4-2. Annual excess cost for VARI in Vietnam.

The tornado diagram (Figure 4-3) shows that the greatest impact on the national cost of VARI management was through the price of antimicrobials and the number of VARI episodes (the national cost varied from US$ 28,307,360 - 52,587,578). The variance of VARI incidence density is associated with a smaller cost variance (from US$ 32,991,714-49,394,375). Variance in the costs of VARI diagnostics, ventilation duration and the proportion of patients with intubation for more than 2 days had minor effects (Figure 4-3).

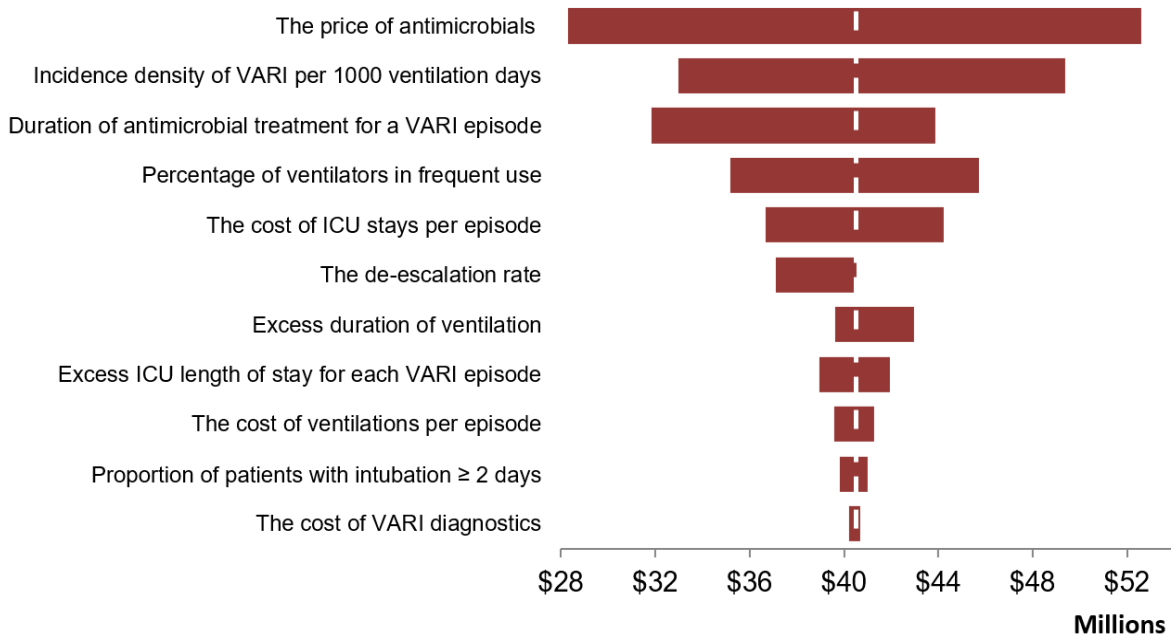


Figure 4-3. Sensitivity of modelled total costs associated with ventilator associated respiratory infection (VARI) diagnosis and treatment in Vietnam.

4.4. Discussion

Our study estimated excess direct medical cost of VARI management across the whole of Vietnam, a lower-middle income country with a high rate of antibiotic resistance and hospital acquired infections. We estimated the excess cost of VARI to the Vietnamese health care system to be tens of millions of US Dollars, a high proportion of which was due to the cost of antibiotics.

The estimation of VARI cost per case in our study is comparable to previous studies in Vietnam but significantly lower than in high-income settings (Stone et al., 2005, Zimlichman et al., 2013). The excess cost of HAI in Vietnam was reported as US$ 865 in a tertiary neonatal intensive care unit (ICU) in 2008 (Ha and Ha, 2012) and US$ 1,131 in tertiary ICU in 2010 (Thi Anh Thu et al., 2015). In a study of 4 ICUs in 3 Vietnamese tertiary referral hospitals from 2013 to 2015, the median ICU related costs associated with admission were US$ 4,723 for patients developing VARI over their stay and US$ 2,534 for those that did not (Phu et al., 2017), corresponding to a cost difference of US$ 2,189. The lack of distinction between the attributed cost before and after VARI is a limitation of a non-matched cohort study (Graves et al., 2010, Phu et al., 2017).

In a recent systematic review of the estimated economic impact of HAI on the US health care system it was shown that the most costly HAI was central line–associated bloodstream

infection (US$ 45,814 in 2012 US$ per case), followed by VAP (US$ 40,144; 95% CI 36,286-44,220) (Zimlichman et al., 2013). The regional or national excess cost for VAP in Europe is estimated to be around US$ 13,000-15,000 per episode (Wyncoll and Camporota, 2012, Arefian et al., 2016a). However, the differences in incomes and healthcare systems make between countries comparisons relatively unedifying. However, given the current Vietnamese per capita health expenditure of US$ 117 (in 2015) (World Health Organization, 2018b), the estimated cost attributed to VARI management (US$ 1174.9) is a high economic burden to the healthcare system in Vietnam (in comparison to the ratio of US per capita health expenditure to attributable cost of US$ 9,536/US$ 40,144 (Zimlichman et al., 2013). These differences in cost may be attributed to differences in costing and the healthcare systems (in Vietnam some healthcare costs are borne 'out of pocket' and these were not included as they are very hard to quantify), similarly some costs for hospital infrastructure are subsidised by the government in Vietnam and many European countries. However, there are also substantially lower costs for human resources in Vietnam and a tendency to use fewer and cheaper disposable items and blood tests.

Our data showed high variability in antimicrobial prices and a high proportion of costs related to antimicrobials (51.1%). In a matched control study of VAP in US, the attributed cost of VAP was US$ 39,828 per episode whilst the cost of antibiotics contributed to US$ 9,367 (23.5%) of the total cost (Kollef et al., 2012). The findings in our study likely relate to disproportionately high antibiotic costs in Vietnam (World Health Organization. Vietnam country office, 2011), very high antibiotic resistance levels and long courses of treatment in Vietnam.

Despite growing healthcare insurance coverage in Vietnam, these high costs of treatment are often not fully covered by insurance. Thus the high cost of VARI may contribute to putting patients and their families into severe debt and act as a barrier to accessing treatment for life threatening conditions (Pekerti et al., 2017). Our study highlights the savings that could be made from even small gains in the reduction of VARI incidence and any safe reduction in the length of therapy for VARI. These are even without potential gains through reducing exposure of the patient and the CCU environment to broad spectrum antibiotics.

Our estimate does not include non-medical direct costs including other out-of-pocket costs incurred by the patients and families for travelling, food and accommodation during

hospitalization and consultations. It also does not include indirect costs associated with loss of income and productivity due to absence from work and premature mortality. Few data on these societal costs are available in Viet Nam. One study estimated that whilst the costs to the health sector averaged US$ 190 and US$ 300 for treating pneumonia and meningitis respectively, in children under 5 years old admitted to a tertiary hospital, the total direct non-medical costs (transportation cost, lodging, soap, diapers, etc) ranged from US$ 50 to US$ 156 and the indirect costs (defined as the productivity loss of caregivers based on the number of working hours lost and monthly income) ranged from US$ 60 to US$ 140 for pneumonia and for meningitis respectively (Le et al., 2014).

One systematic review including three matched cohort studies reported a short attributable ICU length of stay related to VAP (a mean of 6 days, 95% CI, 5.32– 6.87), (Safdar et al., 2005b) whilst one individual participant data meta-analysis from randomised trials assessing VAP prevention measures showed the overall difference of ICU length of stay was 12 days between VAP and non-VAP patients (Melsen et al., 2013). One prospective study in Vietnam showed the difference of ICU stays for VAP and non-VAP was 11 days (Phu et al., 2017). We selected 12 days for our model because it reflects the longer duration of antimicrobial therapy used in Vietnam.

As there are no data for antimicrobial de-escalation rate in Vietnam, we assumed that the de-escalation was similar to studies in US and Europe (Kollef et al., 2006, Alvarez-Lerma et al., 2006). This is likely to represent an overestimate of the proportion that are de-escalated, in part due to limited opportunities to do so because of the high rates of antimicrobial resistance. Our model showed that variation in the proportion of patients de-escalated has a small impact on the total cost of VARI management.

Our study has several limitations. Firstly, resistance rates are increasing and the etiology and susceptibility data used may already be out of date, leading to an under-estimate of the attributable costs. Secondly, as a result of the increasing need for critical care to provide for an aging population with an increasing prevalence of underlying non-communicable diseases, the number of ventilated days nationally may rise leading to underestimation of the number of VARI episodes, in turn underestimating the total national cost of treatment. Furthermore, the studies quoted have focused on the first episode of VARI, potentially underestimating the incidence through exclusion of subsequent episodes. Thirdly, the critical care usage, the capacity of investigations of HAI (including microbiological testing)

and the antibiotic prescription are difficult to extrapolate across Vietnam, as a nationwide CCU registry and quality improvement systems have not been established. Therefore, data on transfer of patients, proportion of patients ventilated for more than 48hrs and antimicrobial prescribing patterns may vary substantially across hospitals.

4.5. Conclusions

Our study found that the direct medical cost for VARI in Vietnam is substantial. Interventions targeting these infections, such as the implementation of infection control and antimicrobial stewardship in critical care units, could provide direct cost savings in addition to their beneficial effect on the generation of antibiotic resistance and should be systematically implemented and evaluated.

Chapter 5: **Impact of MALDITOF diagnostic pathway on optimal antimicrobial therapy**

5.1. Introduction

As shown in Chapter 4, VARI management required microbiological diagnostics for rapid identification of pathogens to make decisions on antimicrobials treatment. In this chapter, we focused on the impact of a novel diagnostic method on antimicrobial use.

In the light of the crisis in antimicrobial resistance, antimicrobial stewardship programs are critical to optimizing antimicrobial use in order to maintain effectiveness, improve patients' safety and minimise costs (MacDougall and Polk, 2005). Techniques in diagnostic microbiology could potentially contribute to stewardship programs through timely bacterial identification and generation of susceptibility profiles, improving appropriate antibiotic selection and reducing broad spectrum antibiotic use.

The rapid detection and identification of a bloodstream pathogen is important to establish the diagnosis of bacteremia and guide treatment. However, blood culture detects pathogens in < 50% of patients with sepsis. Increased time to notification of positive blood culture is associated with increased length of hospitalization (Beekmann et al., 2003). The early, accurate identification of bloodstream infection assists in rapid deployment of optimal antibiotic therapy for these patients with life threatening infections. Clinical microbiological laboratories in resource constrained settings often lack good testing systems and expertise for correct identification of bacterial pathogens. Due to these constraints, time to identification and reporting of bacterial pathogens can be protracted, which may impact on patient care and outcome. Correct and rapid bacterial identification and susceptibility testing is important for providing appropriate antibiotic therapy as soon as possible. A diagnostic system that is robust, accurate, rapid and with low running costs, could be cost saving and improve patient outcome.

Recognising this, investment in hospital laboratory infrastructure and capacity building in LMICs has attracted international attention (Wertheim et al., 2010, Yao et al., 2010, Kinh et al., 2017). The limited resources available need to be used efficiently to develop laboratory capacity optimally, strengthen diagnostics and improve patient outcomes

(Nkengasong et al., 2010). Novel technologies have been developed to improve the speed and accuracy of bacterial diagnosis of aetiology and susceptibility, but many are expensive (Davies et al., 2017, Petti et al., 2006). Most of these technologies have been developed in and for high income countries (Malkin, 2007). Systematic evaluation of the introduction of novel laboratory diagnostics is also necessary in resource constrained settings to show their value in clinical decision-making and quality of care.

MALDITOF-MS can accurately identify bacteria and fungi within a few minutes (Dixon et al., 2015, Scott et al., 2016) at a reasonable cost (excluding machine purchase costs and maintenance) without the same level of expertise needed for standard microbiological diagnostics. Whilst the hardware is expensive (approximately 250,000 USD) it has very low per assay costs (1-1.5 USD/sample) and requires minimal skills (Bizzini and Greub, 2010, Scott et al., 2016, Dixon et al., 2015). Reagents have long expiry dates, unlike traditional biochemical identification systems (leading to regular use of expired reagents is common in LMICs) (Ombelet et al., 2018). Besides the identification of common bacteria, studies have shown that it can also be used for species that are difficult to identify, such as yeasts, anaerobes, and fastidious microorganisms, though a limitation of MALDITOF-MS is mis-identification of some rare specific pathogens due to a limited database (see Supplementary Table 1). Therefore, MALDITOF-MS is an important new technology that is becoming routine in developed countries. As yet there is no robust data exploring the impact of MALDITOF on patient centered outcomes such as mortality and morbidity, and little on stewardship related outcomes such as time to optimal antibiotic therapy and antibiotic costs in resource-limited countries.

Previous studies in high-income settings, where MALDITOF has been combined with an antimicrobial stewardship programme (ASP), have shown clinical impact on reduced time to optimal antibiotic therapy (Huang et al., 2013, Perez et al., 2014), increased proportion of appropriate antibiotic treatment after culture positivity (Vlek et al., 2012) and reduced length of hospital stay (Lin et al., 2016, Perez et al., 2014, Wenzler et al., 2016, Perez et al., 2013). In settings where ASPs were already well established, the results have been less clear.

To date no randomised controlled trials have been performed exploring the benefit of MALDITOF in relation to improving the time to optimal therapy, nor have there been any studies of its use in LMIC settings where antimicrobial stewardship is less well implemented

and the prevalence of resistance is often high. We therefore aimed to determine whether MALDITOF (Maldi Biotyper, Bruker, Germany) reduced the time to OAT compared to standard microbiological identification using VITEK2, API (two bacterial diagnostic systems that require bacterial growth to perform identification) and biochemistry tests in patients with microbiologically confirmed infection in two infectious diseases referral hospitals in Vietnam with limited antimicrobial stewardship.

5.2. Methods

This was a parallel arm randomised controlled trial conducted in 2 tertiary hospitals in Vietnam. The patients were recruited from January and December 2015 among patients with at least one bacterial or fungal pathogen cultured from a normally sterile sample. Eligible samples were blood cultures or aspirates from sterile compartments (cerebrospinal fluid (CSF), deep abscesses, joint fluid, peritoneal fluid, pleural fluid or deep tissue biopsies).

The standard procedure was applied for clinical specimen collection as per the participating hospital's usual practice. The first positive growth of pathogenic organisms was randomly assigned (1:1) to identification by either MALDITOF-MS (Microflex LT/SH, Bruker, Germany, library DB4613) or identification by convention methods, including Gram-staining, API test strips, VITEK2 (bioMérieux, Marcy l'Étoile, France) and other tests as required.

The primary outcome was the proportion of patients, with growth of pathogenic organisms from normally sterile sites, on optimal antimicrobial therapy within 24 hours of positive culture, determined by a blinded independent endpoint review committee. For the formal comparison between the two diagnostic groups, we used a logistic regression model of the primary endpoint depending on the treatment groups, with additional adjustment for the first specimen type (blood vs. other) and the hospital. The main effect measure is an odds ratio of optimal antibiotic treatment within 24 hours between treatment groups and a corresponding two-sided 95% confidence interval (CI) and p-value. Analyses were performed using R (Version 3.4.0). P-values ≤0.05 (two-sided) were considered significant. Full details of the methods are presented in chapter 2.

5.3. Results

5.3.1. Study population

The trial recruited between 1st December 2014 and 15th January 2016. There were 1005 patients with a positive sterile site culture randomised, with 377 patients excluded due to hospital discharge or death prior to the sample becoming positive, or samples growing contaminants. The final analysis included 628 patients, with 326 patients received identifications of pathogens by MALDI-TOF and 302 patients received identifications of pathogens by standard microbiological procedure (Figure 5-1).

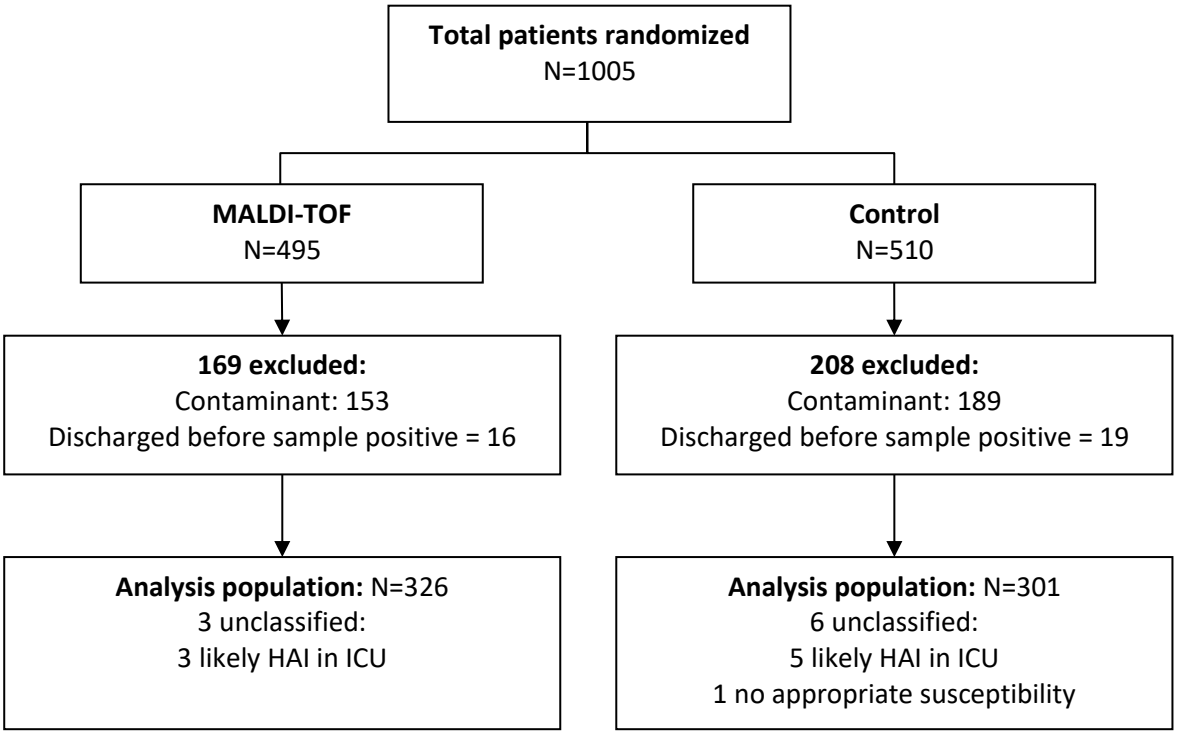


Figure 5-1. Flow chart of participants.

The median age of patients was 47 years old (IQR 32-59) and 64.3% (404/628) were male. Baseline characteristics of patients are shown in Table 5-1 and were not significantly different between the two arms. A quarter of patients (161/628) were admitted to critical care, with the most common sites of infection on randomisation being central nervous system infection (CNSI) (170/628, 27.1%) and intra-abdominal infection (164/628, 26.1%). Among the 628 samples, 421 (67%) were blood, 154 (24.5%) CSF, 46 (7.3%) peritoneal fluid, 6 (1.0%) deep abscess samples, and 1 (0.2%) pleural fluid (Table 5-2).

Table 5-1. Baseline characteristics of patients

	N	MALDITOF-MS n (% or IQR)	N	Control n (% or IQR)	P*
Sex	326		302		0.45
- Female		121 (37)		103 (34)	
Age (median years)		47 (32–59)		47 (35–58)	0.91
Site	326		302		0.57
- Ho Chi Minh city (HCMC)		187 (57)		180 (60)	
- Hanoi		139 (43)		122 (40)	
Source	325		302		0.58
- Direct Admission		163 (50)		144 (48)	
- Hospital transfer		162 (50)		158 (52)	
Ward	326		302		0.41
- Critical Care		79 (24)		82 (27)	
- Other		247 (76)		220 (73)	
Site of infection	326		302		0.33
- Central nervous system (CNS)		81 (25)		89 (29)	
- Abdominal		83 (25)		81 (27)	
- Respiratory		31 (10)		23 (8)	
- Other		40 (12)		44 (15)	
- Unknown		91 (28)		65 (22)	
ICD-10 Code	325		302		0.12
- Sepsis		87 (27)		81 (27)	
- HIV related		69 (21)		59 (20)	
- CNS Infection		46 (14)		49 (16)	
- Cirrhosis		28 (9)		29 (10)	
- Tetanus		6 (2)		4 (1)	
- Other		89 (27)		80 (27)	
Length of illness (median days)	321	6 (3–14)	300	6 (3–14)	0.62
Time from sample collection to first growth (median hours)		34 (22–45)		36 (22–46)	0.41
Time from sample collection to Gram stain (median hours)	287	31 (21–43)	267	33 (20–44)	0.63

Table 5-2. Type of eligible specimen

Specimen type	MALDITOF-MS (N=326) n (%)	Control (N=302) n (%)	P*
Blood Culture	222 (68.1)	199 (65.9)	0.61
Cerebrospinal fluid	75 (23)	79 (26.2)	
Peritoneal fluid	25 (7.7)	21 (7)	
Deep abscesses	4 (1.2)	2 (0.7)	
Pleural fluid	0	1 (0.3)	

In the first observed growth of specimens, 635 bacterial or fungal isolates were obtained (1 patient had 4 isolates in a single culture and 4 patients had 2 isolates). *Enterobacteriaceae* were the most frequently isolates (235/635, 37.0%), followed by Gram positive bacteria (219/635, 34.5%), fungi (105/635, 16.5%) and non-*Enterobacteriaceae* (76/635, 12%). The most common bacterial isolates were *Escherichia coli* (147/635, 23·1%), *Streptococcus suis* (84/635, 13.2%) and *Staphylococcus aureus* (58/635, 9.1%). Methicillin resistance was detected in 31/58 (53.4%) of *S. aureus*, 3rd generation cephalosporin and carbapenem resistance in 113/234 (48.3%) and 10/220 (4.6%) respectively of *Enterobacteriaceae* tested and carbapenem resistance in 10/24 (41.7%) of *Acinetobacter* and *Pseudomonas* species. Aetiology of infection and pattern of drug resistance in selected organism in the two arms is shown in Table 5 3 and Table 5-4.

Table 5-3. Aetiology of infection from the growth of eligible specimen

	MALDITOF-MS (N=329) n (%)	Control (N=306) n (%)	P*
Total Enterobacteriaceae	**131 (40)**	**104 (34)**	**0.15**
- *Escherichia coli*	83 (25)	64 (21)	
- *Klebsiella pneumoniae*	29 (9)	21 (7)	
- Other *Enterobacteriaceae*	19 (6)	19 (6)	
Total Non-Enterobacteriaceae	**40 (12)**	**36 (12)**	**0.98**
- *Acinetobacter* & *Pseudomonas spp*.	14 (4)	12 (4)	
- Other Gram-negatives	26 (8)	24 (8)	
Total Gram-positive	**105 (32)**	**114 (37)**	**0.18**
- *Streptococci*	70 (21)	85 (28)	
- *Staphylococcus aureus*	32 (10)	26 (8)	
- Other Gram-positives	3 (1)	3 (1)	
Total Fungi	**53 (16)**	**52 (17)**	**0.85**
- *Cryptococcus neoformans*	29 (9)	34 (11)	
- *Talaromyces marneffei*	20 (7)	16 (5)	
- Other fungi	4 (1)	2 (1)	

Table 5-4. Bacteria resistance profiles (where tested)

Resistance profile	MALDITOF-MS n/N (%)	Control n (%)
S. aureus with methicillin resistance	16/32 (50)	15/26 (58)
Enterobacteriaceae with 3G-C resistance	63/131 (48)	50 /103[a] (49)
Enterobacteriaceae with carbapenem resistance	9/123[b] (7)	1/97[c] (1)
Acinetobacter or *Pseudomonas* species with carbapenem resistance	5/13[d] (38)	5/11[e] (45)
Enterococci with vancomycin resistance	1/5 (20)	2 (33)

[a] 1 isolate not tested (*K. pneumoniae*)

[b] 8 isolates not tested (*1 Klebsiella pneumoniae*, 7 *Salmonella* spp.)

[c] 7 isolates not tested (2 *E. coli*, 2 *K. pneumoniae*, 3 *Salmonella* spp.)

[d] 1 isolate not tested (*Acinetobacter sp.*)

[e] 1 isolate not tested (*Acinetobacter baumannii*)

5.3.2. Primary outcome

The proportion of patients who received optimal therapy within 24 hours, was not different between MALDITOF-MS (135/326, 41.4%) and control arms (119/301, 39.5%) (Adjusted Odds Ratio (AOR) 1.17; 95% confidence interval (CI) 0.82-1.68, p=0.38) (Table 5-5). In 9 cases (3 MALDITOF-MS, 6 control arm) the review committee recorded therapy as 'unclassifiable' and these were included in the 'not optimal' outcome per the analysis plan. The predominant reason for the committee to consider a treatment non-optimal was because therapy was too broad (252/373, 67.6%) (Table 5-6). The proportion of optimal therapy in predefined subgroups was not significantly different between the MALDITOF-MS and control arms (Table 5-7).

Table 5-5. Proportions of patients on optimal antibiotic therapy within 24 and 48 hours of growth

	MALDITOF-MS n/N (%)	Control n/N (%)	AOR (95% CI)a	p
Within 24 hours	135/326 (41.4)	119/301 (39.5)	1.17 (0.82 – 1.68)	0.38
Within 48 hours	151/326 (46.3)	140/301 (46.5)	1.05 (0.74 – 1.51)	0.77

[a] Adjusted odds ratio adjusted for specimen type (blood/other) and site

Table 5-6. Reasons for non-optimal therapy at 24 hours after culture growth

	MALDITOF-MS (N=191) n (%)	Control (N=182) n (%)	p
Pathogen not covered	54 (28.3)	46 (25.3)	0.514
Therapy too broad	130 (68.1)	122 (67)	0.832
Therapy potentially effective but not ideal	3 (1.6)	6 (3.3)	0.328
Growth of second pathogen within 48 hours that was not covered	1 (0.5)	2 (1.1)	0.615
No data	3 (1.6)	6 (3.3)	0.328

Table 5-7. Pre-specified subgroup analyses of proportion of patients on optimal therapy within 24 and 48 hours of culture growth

	Proportion optimal within 24 hours of culture growth						Proportion optimal within 48 hours of culture growth					
	N	MALDITOF	N	Control	AOR (95% CI)[a]	P	N	MALDITOF	N	Control	AOR (95% CI)[a]	P
		n (%)		n (%)				n (%)		n (%)		
Sample Type						0.45[b]						0.60[b]
Blood	222	75 (33.8)	199	59 (29.6)	1.31 (0.84 – 2.07)	0.23	222	88 (39.6)	199	76 (38.2)	1.14 (0.73 – 1.78)	0.56
Other	104	60 (57.7)	102	60 (58.8)	0.97 (0.54 – 1.74)	0.92	104	63 (60.6)	102	64 (62.7)	0.92 (0.51 – 1.67)	0.79
Site						0.61[b]						0.96[b]
HCMC	187	108 (57.8)	179	100 (55.9)	1.10 (0.72 – 1.68)	0.66	187	122 (65.2)	179	115 (64.2)	1.05 (0.68 – 1.63)	0.81
Hanoi	139	27 (19.4)	122	19 (15.6)	1.38 (0.71 – 2.74)	0.34	139	29 (20.9)	122	25 (20.5)	1.06 (0.57 – 1.99)	0.86
Pathogen type						0.62[b]						0.49[b]
Gram-positive	103	45 (43.7)	111	41 (36.9)	1.74 (0.90 – 3.42)	0.1	103	47 (45.6)	111	47 (42.3)	1.42 (0.74 – 2.74)	0.3
Gram-negative	167	58 (34.7)	137	47 (34.3)	1.09 (0.65 – 1.82)	0.75	167	70 (41.9)	137	62 (45.3)	0.91 (0.55 – 1.52)	0.72
Fungi	52	31 (59.6)	51	31 (60.8)	1.11 (0.41 – 3.07)	0.83	52	33 (63.5)	51	31 (60.8)	1.38 (0.53 – 3.77)	0.51
Admitted from						0.92[b]						0.73[b]
Home	163	71 (43.6)	144	61 (42.4)	1.17 (0.72-1.90)	0.53	163	80 (49.1)	144	71 (49.3)	1.10 (0.68-1.79)	0.7
Hospital	162	64 (39.5)	157	58 (36.9)	1.17 (0.68-2.01)	0.57	162	71 (43.8)	157	69 (43.9)	1.00 (0.58-1.70)	0.99
Final diagnosis						0.71[b]						0.74[b]
Meningitis	76	57 (75.0)	64	47 (73.4)	1.3 (0.56 – 3.04)	0.54	76	59 (77.6)	64	50 (78.1)	1.12 (0.47 – 2.69)	0.79
Other	250	78 (31.2)	237	72 (30.4)	1.07 (0.71 – 1.63)	0.74	250	92 (36.8)	237	90 (38.0)	0.96 (0.64 – 1.45)	0.86

[a] Adjusted for site and specimen type except where these are part of the subgroup.

[b] Test for heterogeneity.

5.3.3. Secondary outcomes

There was no difference in the proportion of patients on optimal therapy within 48 hours of growth between MALDITOF-MS (151/326, 46.3%) and control arms (140/301, 46.5%, AOR 1.05 p=0.77) (Table 5-7) or in the time from growth to optimal antimicrobial therapy (HR 0.992 (95%CI 0.808– 1.217) p=0.937) (Figure 5-2).

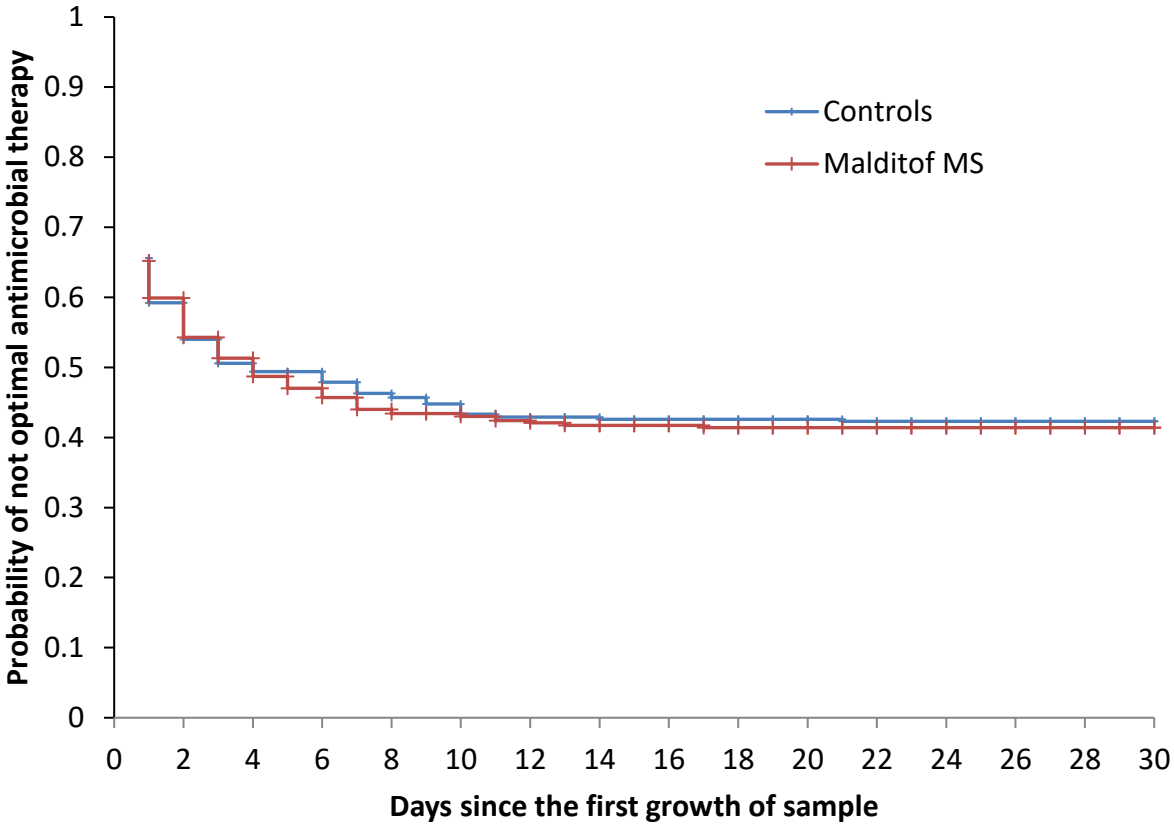


Figure 5-2. Time from growth to not optimal antimicrobial therapy (OAT).

There was no difference in the ordinal outcome (hospital outcome grouped into 5 categories - death, palliative discharge, survived with sequelae, transferred to another hospital and recovered) adjusted for site and sample type, between the MALDITOF-MS and control arms (AOR 0.869 (95%CI 0.65 – 1.16) p = 0.34). Although median hospital stay was the same for both arms, Cox proportionate hazards adjusted for site and specimen type demonstrated an increased hazard ratio for hospital discharge in the MALDITOF-MS arm Figure 5-3. Analysis in survivors only showed similar median length of stay in the MALDITOF-MS (15 days, IQR 11-21) and control arms (16 days, IQR 11-23). There was no significant difference in other pre-specified secondary outcomes (Table 5-8).

Table 5-8. Pre-specified secondary patient-level outcomes

Secondary outcomes	N	MALDITOF-MS Median (IQR)	N	Control Median (IQR)	P
DDD of antimicrobial consumption from enrolment to discharge	304	16.0 (8.4–33)	283	18.0 (9.0–39.3)	0.35[a]
Days of antimicrobial therapy from enrolment to discharge	304	10 (6–15)	282	11 (7–17.8)	0.287[b]
Days in hospital	322	15 (10–21)	301	16 (10–23)	0.039[c]
Days spent in Critical Care	115	5 (2–11)	100	4 (3–10.3)	0.38[d]
Hours from first growth to pathogen identification	324	2.2 (1.7–28.1)	298	26.6 (24.7–48)	ND
Hours from sample collection to pathogen identification	324	43.2 (28.1–69.1)	298	66 (48–88.6)	ND
Days from sample collection to hospital discharge	326	11 (6.1–16.3)	302	12.3 (7.2–19)	ND

a Linear regression coefficient 0.92 (95% CI 0.78–1.09) after adjustment for site and specimen type.

b Hazard ratio for stopping antibiotics 1.09 (95% CI 0.92–1.29) after adjustment for site and specimen type.

c Hazard ratio for hospital discharge 1.18 (95% CI 1.01–1.38) after adjustment for site and specimen type.

d Hazard ratio for ICU discharge 1.13 (95% CI 0.86–1.49) after adjustment for site and specimen type in those that had an ICU stay

ND statistical comparison not performed (as stipulated in the analysis plan).

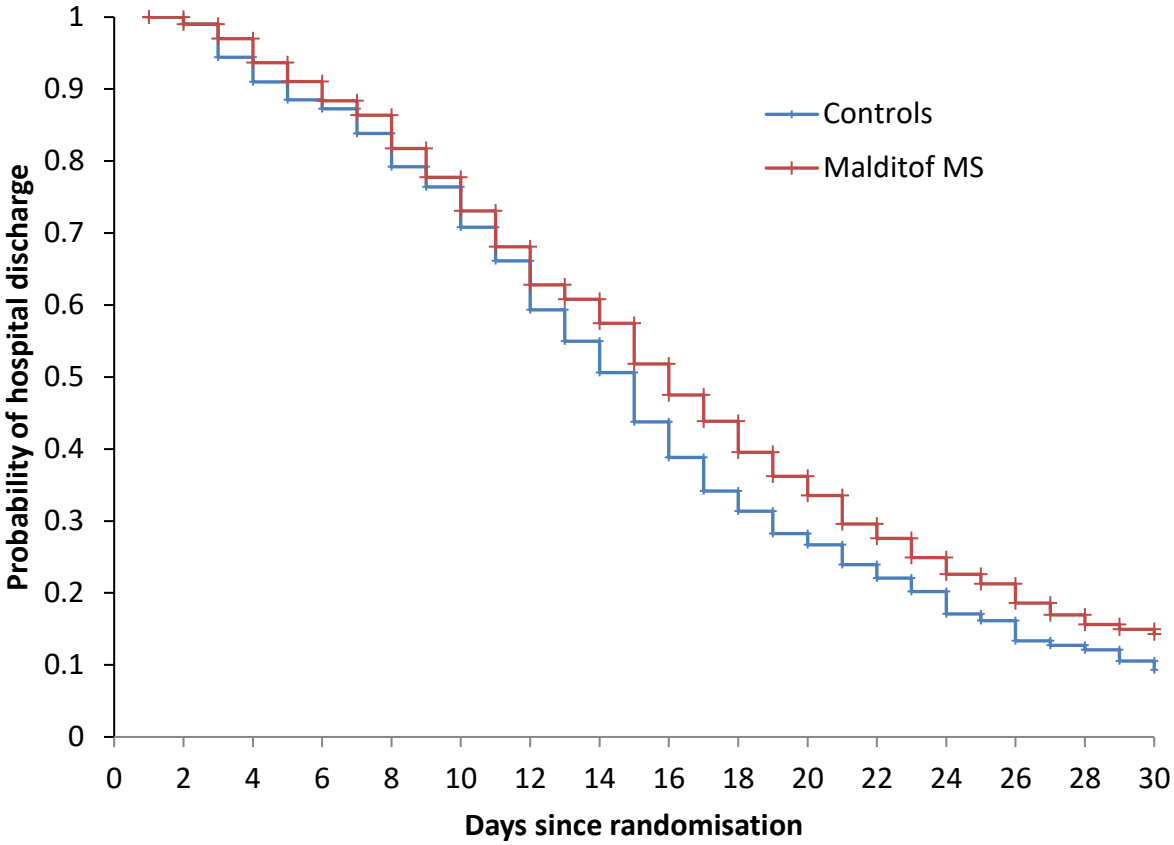


Figure 5-3. Time from randomisation to hospital discharge.

5.3.4. Subgroup analyses

Limiting the analysis to the subgroup of patients with Gram positive organisms cultured showed a trend towards an increased proportion on optimal therapy at 24 hours in the MALDITOF-MS arm (45/103, 43.7%) compared to the control arm (41/111, 36.9%; p=0.1). However, there was no significant effect observed in any pre-specified subgroup analysis (Table 5-8).

5.3.5. Exploratory analyses

An analysis of mortality as a binary outcome (death or palliative discharge compared with all other outcomes) adjusted for site and sample type revealed no difference (MALDITOF-MS arm 52/326 (16.0%), control arm 43/301 (14.3%), AOR 1.13 (95%CI 0.73 – 1.76, p=0.59)).

Analysis of time spent in intensive care, amongst those that were admitted to ICU at any time during their admission, showed no difference between the MALDITOF arm

(median time in ICU 5 days, IQR 2 – 11) and the standard diagnostics arm (median time in ICU 4 days, IQR 3 – 10.2), (Cox proportionate hazards ratio for ICU discharge adjusted for site and specimen type for ICU discharge 1.13 (95%CI 0.859 – 1.49, p = 0.38)). There remained no significant difference in the hazard ratio for ICU discharge when the analysis was limited to exclude outliers or limited to survivors (HR 1.19, 95%CI 0.9-1.576, p=0.221). Excluding outliers (the longest staying 1% of patients) in an analysis of hospital stay to explain the increased hazards ratio for hospital discharge in the MALDITOF-MS arm, the hazard ratio for discharge in the MALDITOF-MS arm dropped to 1.16 (p=0.067).

An analysis to determine whether results were reaching the wards more quickly in the MALDITOF-MS arm demonstrated that the median time from growth to the pathogen identification report being received on the ward was 10.1 hours (IQR 1.9 – 32.9 hours) in the MALDITOF-MS arm and 31.0 hours (IQR 27.4 – 54 hours) in the control arm.

A subgroup analysis of the 146 patients not on optimal therapy at the time of culture growth, that subsequently did receive optimal therapy, showed that the median time to optimal therapy was 2.0 (IQR 0.7 – 5.6) days in the MALDITOF-MS arm and 2.6 (IQR 1.2 – 4.8) days in the control arm. A further subgroup analysis of those patients according to whether they were in critical care or not when cultures were drawn showed no significant difference in both those in critical care (OR 0.90 (95% CI 0.5 – 1.72, p = 0.81)) or other wards (OR 1.16 (95%CI 0.79–1.69, p = 0.45)).

An analysis looking at the proportion receiving an antibiotic with no activity to the pathogen grown per antibiotic susceptibility testing (AST) results (inadequate therapy) showed little difference between the two arms (54/326, 16.6% and 46/302, 15.2% in MALDITOF-MS and control arms respectively).

The median total antibiotic and antifungal consumption in defined daily doses (DDD) from enrolment to hospital discharge was similar between the MALDITOF (15.5; IQR 8.0 – 33.0) and control arms (18·0; IQR 8.1 – 39.5), linear regression coefficient 0.94 (95% CI 0.79 – 1.12, p=0.482 after adjustment for site and specimen type; data only available for 294 and 273 patients in MALDITOF and standard diagnostic arms respectively). Similarly, the median total duration of antibiotic or antifungal therapy from enrolment to hospital discharge was similar in both arms (10 days, IQR 6– 15 days in MALDITOF and 11 days, IQR 7 – 18 days in control arm, hazard ratio for stopping antibiotics 1.09 (95% CI 0.93 – 1.29), p=0·287 after adjustment for site and specimen type; data only available for 282 and 304

patients in MALDITOF and control arms respectively). Restriction of these data to survivors did not result in a significant difference between MALDITOF (11; IQR 7-16) and standard arms (11, IQR 8-18), HR= 1.08 (95% CI 0.91 – 1.29), p=0.387.

There was no significant difference between the hospital costs between the MALDITOF (median cost 16,323 USD, IQR 7,824 – 31,252) and control arms (15,928 USD, IQR 7,125 – 33,175, p=0.641). Costs for microbiology testing were also similar between MALDITOF (median cost 798 USD (IQR 528 – 1,200) and the control arm (759 USD (IQR 480 – 1,104), p= 0.120. Data on costings were only available for 297 patients in the MALDITOF arm and 269 in the control arm.

5.4. Discussion

Our study demonstrates that early identification of pathogens from cultures of blood and other sterile sites using MALDITOF-MS did not result in a difference in the proportion of patients on optimal therapy within 24 hours of first growth. Neither did MALDITOF-MS alter the proportion on optimal therapy by 48 hours, the time taken to provide optimal therapy, the duration or total antibiotic therapy, patient outcomes or time in intensive care. We found an association between MALDITOF-MS and earlier hospital discharge, but the significance was removed when outliers were excluded (very long stay patients) and we therefore consider it unlikely to be of clinical significance.

Using rapid Gram staining of blood culture and notification in a matched case control study, quick Gram stain turnaround time (<1 hour) was associated with significantly lower mortality (10.1% vs. 19.2%) (Barenfanger et al., 2008). Introduction of automated identification systems (such as Vitek, MicroScan WalkAway or Phoenix) has offered advantages in early reporting of pathogen identification and antimicrobial susceptibilities. In a historical cohort study in all cultures from 2 periods in 1997 at a center in the United States using the Vitek system, quick turnaround time for the reporting of antibiotic susceptibility testing facilitated early appropriate antibiotic therapy initiation (94% vs 77% for normal manner), reduced length of stay by 2 days and reduced cost by $1,750 per patient (Barenfanger et al., 1999). In a randomized controlled trial of 1498 patients in the Netherlands with positive growth in any sterile bodily fluid (excluding urine), the intervention group of Vitek 2 (next generation platform) showed significant reduction in antibiotic use (6 defined daily doses lower per patient, p=0.012), and quicker antibiotic switches than in the control arm, but mortality (17.6% versus 15.2%, p=0.21) was not

significantly reduced in comparison to the control arm of Vitek 1 system (Kerremans et al., 2008). However the effectiveness of MALDITOF-MS in reducing antibiotic use has not been explored in randomized control trials although MALDITOF-MS may have better performance than Vitek-2 in identifying pathogens in routine clinical isolates (Guo et al., 2014). In a study among 155 episodes of peritoneal dialysis-related peritonitis, dialysate effluent fluid culture was identified by MALDI-TOF (57 isolates) and conventional standard method (98 isolates). MALDI-TOF reduced the time to identification of causative pathogens by an average time of 37-68 hours and shortened the length of hospital stay (5.2 ± 4.8 versus 8.2 ± 4.5, p = 0.001) (Lin et al., 2016). However, the impact on targeting antibiotic therapy or antibiotic use was not reported.

The rapid identification of pathogens in combination with antibiotic stewardship program in high income countries was seen to significantly reduce the time to targeted antibiotic therapy, unnecessary use of antibiotics for contaminated samples, overall length of stay and hospitalization cost, but mortality was not significantly reduced (Box et al., 2015). This was seen in a multicenter, pre-post, quasi-experimental study using an automated nucleic acid system (Verigene Gram-Positive Blood Culture Test) combined with antibiotic stewardship conducted by infectious diseases physicians and pharmacists (Box et al., 2015). Similar designs with MALDITOF-MS and antibiotic stewardship were implemented among patients with bacteremia and candidemia and showed an improved time to optimal antibiotic therapy and mortality reduction in some studies (Huang et al., 2013, Perez et al., 2014) but not in others (Wenzler et al., 2016, Perez et al., 2013). The impact of identification by MALDITOF-MS on clinical outcome in previous studies was summarised in Supplementary Table 2.

De-escalation is a component of antimicrobial stewardship to replace empirical broad-spectrum by a narrower antimicrobial therapy and shorten the duration of antimicrobial (Dellit et al., 2007). It is practically done by subjectively reviewing the patient's microbial culture results, using a ranking system based on spectrum of antimicrobial activity (Leone et al., 2014) or using quantitative score (Madaras-Kelly et al., 2014). In a study on 123 physicians and pharmacist to measure the spectrum of antimicrobial activity by a numerical spectrum score and defining de-escalation, the optimal time after initial antibiotic therapy to de-escalate therapy was 24 hours at individual perspective (63% of participants), 72 hours and 96 hours at facility-level perspective in 44% and 42% of participants respectively

(Madaras-Kelly et al., 2014). To explore the potential barriers in antimicrobial de-escalation, I performed informal interviews with doctors in non-study hospitals to reveal explanatory factors associated with this. The reasons for not switching to optimal antimicrobial therapy was listed as doctors' concerns about safety and quality of antimicrobials, especially the older or more narrow spectrum antibacterials and generic drugs, which are only procured by domestic manufactures or manufactured in other LMICs. Another barrier was reported as doctors' anecdotal belief that intravenous antimicrobials have better bioavailability and effectiveness (usually contrary to evidence), and therefore they prefer to not switch to an alternative of narrow spectrum antimicrobials or an oral formulations of the same drug.

In common with previous studies, we found quicker pathogen identification and reporting. Our study gives some indication as to why MALDITOF-MS results did not result in improved outcomes. In both arms Gram stain results for positive blood cultures were available rapidly and possibly already provided sufficient information. The most common cause of suboptimal therapy was use of excessively broad therapies, suggesting that there were delays in de-escalation of therapy. There was some evidence that the intervention was more successful in patients with Gram positive infections. This may relate to identifying *Streptococcus suis*, a common cause of both meningitis and severe sepsis which has always been susceptible to penicillin (Huong et al., 2014) and exclusion of listeriosis through identification of an alternative pathogen.

It has been suggested that research on ASP should evaluate its effectiveness on healthcare provider and patient centred outcomes, as well as microbiological ones (McGregor and Furuno, 2014). Rapid diagnostics, including MALDITOF-MS, in combination with ASPs have been proposed to optimise therapy faster and improve clinical outcomes (Barlam et al., 2016). In a meta-analysis of the effectiveness of test result notification to clinicians on targeted therapy for patients with bloodstream infection, it was suggested that a combination of rapid molecular or phenotypic methods with result notification may be more effective than traditional identification techniques in shortening delays in achieving targeted therapy (Buehler et al., 2016). However, ASPs require resources that are often lacking in low- and middle-income settings. Our study and others indicate that the process of reporting and acting on results is likely to be as important as the speed with which results are generated.

There have been few randomised controlled trials exploring the impact of these technologies (Holtzman et al., 2011, Banerjee et al., 2015) and no such evidence for MALDITOF is available at the time of writing. One trial of MALDITOF-MS compared with conventional microbiology with 28 day mortality as the primary endpoint has completed recruitment in the UK but has yet to be reported (the RAPIDO trial, https://doi.org/10.1186/ISRCTN97107018). Whilst studies have established that MALDITOF-MS can identify pathogens in the tropics (Lo et al., 2015) (see Supplementary Table 1), all eight publications that explored the clinical impact of MALDITOF-MS (Vlek et al., 2012, Huang et al., 2013, Lin et al., 2016, Osthoff et al., 2017, Wenzler et al., 2016, Perez et al., 2013, Perez et al., 2014, Lockwood et al., 2016) were conducted in HICs (4 USA, 1 Taiwan, 1 The Netherlands, 1 Switzerland). Three studies explored the impact of MALDITOF-MS compared with conventional diagnostics (Lin et al., 2016, Osthoff et al., 2017, Vlek et al., 2012) without an ASP component. One was restricted to peritoneal dialysis fluid (Lin et al., 2016), showing a significant reduction in hospital stay. The others recruited patients with bloodstream infections (Osthoff et al., 2017, Vlek et al., 2012). One showed a significant improvement in the proportion with appropriate therapy within 24h of growth (from 64% to 75.3%, p=0.01)(Vlek et al., 2012), whilst the other found a non-significant difference in the proportion of patients receiving active treatments within 48 hours of blood cultures being drawn between the MALDITOF-MS (95.6%) and control arms (89.8%, p=0.09) (Osthoff et al., 2017). Five studies examined the impact of MALDITOF-MS plus ASP with conventional diagnostics without ASP (Huang et al., 2013, Lockwood et al., 2016, Perez et al., 2013, Perez et al., 2014, Wenzler et al., 2016). These studies all showed improvements in time to active or appropriate therapy and two showed improvements in mortality (Huang et al., 2013, Perez et al., 2014).

Our study has the advantage of addressing the single intervention of MALDITOF diagnostics, thereby highlighting the need to investigate the addition of other supports, such as ASP, if clinically significant impacts are to be achieved. It is consequently not generalisable to settings where ASP interventions are already in place, where studies in HICs have shown good results. The individually randomised nature of the study is robust but a potential limitation is that this design may not account for changes in prescribing that could arise from a 'cultural shift' resultant from a wholesale change in diagnostic practice. Although the use of two sites and the large sample size is a strength, the use of specialist infectious diseases hospitals could cause bias and poor generalisability. However, it seems

unlikely that MALDITOF-MS alone would be more effective at changing prescribing practice in a setting where staff are less experienced in managing infection and the uniformity of the results across different pathogen groups makes it unlikely that the case mix seen is responsible for the negative results. Our study did not achieve the prespecified sample size. The discrepancy being due to a higher than anticipated number of patients having contaminated cultures. However, this large sample size was determined to accurately assess if the intervention was effective for blood cultures in each hospital, we surpassed the sample size necessary for the primary outcome and it is thus unlikely we missed a relevant positive result. Our data relate to a setting where antibiotic resistance is particularly high and may not be generalisable to LMIC settings where resistance rates are lower. The high contamination rate during the study was noted during the study. Whilst there was a desire to avoid undue interference with hospital practice, attempts were made to reduce this through additional education for those responsible for bloodletting, replacement of liquid disinfection fluids with disposable sterile alcohol wipes and ongoing audit and feedback. As a limitation of this study, we were not able to report the exact time of identified pathogen and antimicrobial susceptibility testing results reported to the doctor due to inconsistent and incomplete reporting: the time was often reported in days rather than hours.

Despite these negative findings there are several positive aspects to MALDITOF-MS that should not be overlooked. Firstly, even speedier identification can be achieved by both processing samples direct from blood culture (La Scola and Raoult, 2009) (without the subculture onto blood agar) and by running the machine more frequently than twice daily. However, this requires changes to work flow that were not possible within the trial and, based on the results we obtained, would be unlikely to have had an impact on the results. Rapid AST, either through short incubation with antibiotics or through analysis of the spectra obtained has also now been described using MALDITOF-MS (Lange et al., 2014). Though more technically difficult, such results may have been more compelling in this setting and warrant further evaluation. We planned to analyse the cost effectiveness of MALDITOF-MS, however neither hospital changed their pricing structure dependent on the diagnostic modality and we were thus unable to. Finally, rapid detection of contaminants (not explored in this study) can result in reductions in antibiotic use, save laboratories time and money and increase the throughput of the labs.

5.5. Conclusion

Our study showed no improvement in antimicrobial prescribing or other patient or provider centred outcomes through MALDITOF-MS. MALDITOF-MS did produce results rapidly in our setting. Our findings suggest that whilst MALDITOF-MS may have other compelling advantages, it is unlikely to lead to improvements in prescribing without investment in ASPs and education of the diagnostic and prescribing workforce.

Chapter 6: **Hospital acquired blood stream infection in intubated patients in 3 Vietnamese ICUs**

6.1. Introduction

In chapter 5, I showed that advanced technology of microbiological diagnostics alone did not improve antimicrobial use in patients with bloodstream infections (BSI). In this chapter, we examined the diagnosis of BSI in a prospective randomised controlled trial to measure the burden of hospital acquired BSI in critical care units in Vietnam.

Since their first introduction in 1929 (Sette et al., 2012), intravascular catheter placement has been a very common procedure in ICUs, with a rate of arterial catheter and central venous catheter use ranging from one-third to nearly all patients in ICUs (Gershengorn et al., 2014). Intravascular catheters are used for different indications, including fluid replacement, transfusion, parenteral nutrition, antimicrobial therapy, monitoring of hemodynamic status and hemodialysis (Mermel et al., 2009). However, the use of intravascular catheters is associated with a risk of intravascular catheter-related bloodstream infections (CRBSI), caused by microorganisms that colonise the catheter or contaminate the fluid administered through the catheter. These are mostly coagulase-negative staphylococci, *S. aureus*, *Candida* species, and enteric Gram-negative bacilli (Mermel et al., 2009). Central line-associated BSI often ranked third among the most common HAIs. In ICUs in LMICs, the incidence density of catheter-related bloodstream infections was 1.7 – 44.6 per 1000 catheter-days whilst for catheter-related urinary-tract infections this was 1.4 – 23.0 per 1000 urinary catheter-days and for ventilator-associated pneumonia 3.2 – 56.9 per 1000 ventilation-days (Allegranzi et al., 2011).

The burden of HAIs, including specific type of HAIs in SEA is not well documented (Ling et al., 2015). The incidence density by specific type of HAIs in SEA was 14.7 per 1000 ventilator-days (95% CI, 11.7–17.7) for ventilator-associated pneumonia, 4.7 per 1000 catheter-days (95% CI, 2.9–6.5) for central line–associated bloodstream infection and 8.9 per 1000 catheter-days (95% CI, 6.2–11.7) for catheter-associated urinary tract infection (Ling et al., 2015). In a meta-analysis of studies from 2009 to 2016, patient's characteristics on admission including diabetes (relative risk (RR), 1.76; 95% CI, 1.27-2.44), immunosuppression (RR, 1.24; 95% CI, 1.04-1.47), and body temperature (mean difference

(MD), 0.62; 95% CI, 0.41-0.83) and treatment-related factors, including surgery time (mean difference 34.53; 95% CI, 22.17-46.89), reoperation (RR, 7.94; 95% CI, 5.49-11.48), exposure to cephalosporin (RR, 1.77; 95% CI, 1.30-2.42), days with central venous catheter (MD, 5.20; 95% CI, 4.91-5.48), intensive care unit (ICU) admission (RR, 3.76; 95% CI, 1.79-7.92), days of ICU stay (MD, 21.30; 95% CI, 19.81-22.79), and mechanical ventilation (OR, 12.95; 95% CI, 6.28-26.73) were associated with HAIs (Rodriguez-Acelas et al., 2017).

In a prospective observational study in the Hospital for Tropical Diseases in southern Vietnam, the incidence density of HAIs in ICU were 23/1000 patient days and pneumonia was the most common HAIs (49.1%) followed by UTIs (36.8%) and BSI (14.1%) (Thuy et al., 2018). Whilst the incidence density of VARI was quite consistent among studies, the data of CRBSI in Vietnam was limited. A point prevalence study in ICUs in 14 provincial and national level hospitals in the VINARES network in Vietnam showed a HAI prevalence of 29.5% (965/3266). The majority (79%, 804) were hospital acquired pneumonia, with 73% (589) being ventilator associated. HABSI ranked second with 44 cases (4.4%), 27 of which (61.3%) were catheter associated (Phu et al., 2016).

This chapter aims to estimate the incidence density rate of HABSI (which is the ratio of the number of cases to the total time the patient population is at risk of BSI and presented in per 1000 patient-days) and factors associated with CRBSI in intubated patients in ICUs in Vietnam using data from patients enrolled in the trial to study the effects of continuous endotracheal cuff pressure control on preventing ventilator associated respiratory infections (VARI) in newly intubated patients (Chapter 2)

6.2. Methods

6.2.1. Study population

This study used data from an open-label randomised controlled trial (RCT) to study the effects of continuous endotracheal cuff pressure control on preventing VARI in newly intubated patients (Chapter 2). Six hundred patients aged 18 years and older who were admitted to the ICUs in 3 hospitals in Vietnam were included and randomised in control group (manual cuff pressure control) or intervention group (automated cuff pressure control). The patients in both arms received diagnostics for hospital acquired infections when a new antimicrobial was started or the treating doctor suspected a new infection. All patients were followed during hospitalisation and at 30 days and 90 days after

randomisation. In this chapter, patients from both assigned groups were included in the analysis. Details on the methods are presented in Chapter 2.

6.2.2. Case definitions of hospital acquired bloodstream infection

Hospital acquired bloodstream infection (HABSI) were defined as the first episode of a new infection in a patient admitted to ICU for at least 48 hours by the European network for the surveillance of healthcare-associated infections (European surveillance of healthcare-associated infections in intensive care units, 2015). Bloodstream infection was defined as a patient having at least one positive blood culture for a recognised pathogen OR a patient having at least one of the following signs or symptoms: fever (> 38 °C), chills, or hypotension and two positive blood cultures for a common skin contaminant (coagulase-negative staphylococci*, Micrococcus spp., Propionibacterium acnes, Bacillus spp., Corynebacterium spp.*) (from two separate blood samples, usually within 48 hours) (European surveillance of healthcare-associated infections in intensive care units, 2015). Central line-associated BSI (CLABSI) was defined as a patient with a laboratory confirmed bloodstream infection and an central venous line (subclavian veins, internal jugular veins or femoral veins) present on the date of laboratory confirmed BSI or the day before (Centers for Disease Control and Prevention/ National Healthcare Safety Network, 2019).

6.2.3. Statistical Analysis

The incidence density rate of hospital acquired bloodstream infection in ICU was calculated by dividing the number of cases with HABSI by the total number of patient-ICU days and multiplying with 1000 (per 1000 patient-days). The incidence density rate of a CLABSI was calculated by dividing the number of CLABSI by the number of patient-days with central venous catheter during ICU stays (per 1000 device days (Centers for Disease Control and Prevention/ National Healthcare Safety Network, 2019).

All data were analysed using R (Version 3.6.2). Factors associated with HABSI were compared between patients with and without HABSIs using Cox's proportional hazards regression. Variables were selected by review of the literature (demographic, severity, interventions age) and were included in Cox proportional hazards model. All tests were two-tailed and differences were considered statistically significant at p values ≤0.05.

Table 6-1. Participant's characteristics

Characteristics	All patients n=591	Patients with HABSI (n=44)	Patients without HABSI (n =547)	P value
Male sex (n, %)	408/591 (69.0%)	34/44 (77.3%)	374/547 (68.4%)	0.219
Age (years), (median, IQR)	58.0 (42.0-70.0)	60.0 (43.0-74.2)	58.0 (42.0-70.0)	
Place of enrolment (n,%)				0.922
TVH	135/591 (22.8%)	10/44 (22.7%)	125/547 (22.9%)	
HCM	294/591 (49.7%)	23/44 (52.3%)	271/547 (49.5%)	
NHTD	162/591 (27.4%)	11/44 (25.0%)	151/547 (27.6%)	
Transferred from other hospital (n,%)	422/591 (71.4%)	30/44 (68.2%)	392/547 (71.7%)	0.623
Central venous catheter placement (n,%)	333/591 (56.3%)	33/44 (75.0%)	300/547 (54.8%)	0.001
Arterial catheter placement (n,%)	167/591 (28.3%)	22/44 (50.0%)	145/547 (26.5%)	<0.001
Charlson Comorbidity Index (n,%)				0.143
0	361/591 (61.1%)	28/44 (63.6%)	333/547 (60.9%)	
1-2	133/591 (22.5%)	5/44 (11.4%)	128/547 (23.4%)	
3-4	44/591 (7.4%)	6/44 (13.6%)	38/547 (6.9%)	
≥5	53/591 (9.0%)	5/44 (11.4%)	48/547 (8.8%)	
APACHE II score at enrolment (median, IQR)	17.0 (13.0-22.0)	16.0 (12.8-22.0)	18.0 (14.0-22.0)	0.245
Cause of admission (n,%)				
Tetanus	180/591 (30.5%)	22/44 (50.0%)	158/547 (28.9%)	0.003
Pneumonia (any)	167/591 (28.3%)	8/44 (18.2%)	159/547 (29.1%)	0.123
Sepsis/septic shock	141/591 (23.9%)	6/44 (13.6%)	135/547 (24.7%)	0.098
CNS Infection	127/591 (21.5%)	5/44 (11.4%)	122/547 (22.3%)	0.089
COPD	10/591 (1.7%)	0/44 (0.0%)	10/547 (1.8%)	0.366
Cerebrovascular disease	9/591 (1.5%)	0/44 (0.0%)	9/547 (1.6%)	0.391
Myocardial infarction	27/586 (4.6%)	1/44 (2.3%)	26/542 (4.8%)	0.442
Others	66/591 (11.2%)	2/44 (4.5%)	64/547 (11.7%)	0.147

6.3. Results

6.3.1. Demographic and clinical characteristics of the patients

From November 2016 to December 2018, we recruited a total of 597 patients for the randomised controlled trial of the effectiveness of continue cuff pressure control and 591 patients who had been hospitalised for more than 48 hours were included in this analysis. The median age of patients was 58 years (IQR 42-70) and most were male (408/591 or 69%). The demographics of patients were summarised in Table 6-1. Of the patients studied, all patients were intubated at the time of enrolment, 153/591 (25.9%) had a tracheostomy at randomisation, 318/591 (53.8%%) had central venous catheters (CVC), and 153/591 (25.9%) had an arterial catheter during their ICU stay.

6.3.2. Incidence density rate of hospital acquired bloodstream infection

Among 591 patients who were admitted to ICUs, 44/591 patients (7.4%) had at least one episode of HABSI. There were 333/519 patients with central line placement and a total number of central catheter days of 3677. Thirty-one patients with HABSI (31/44 or 70.5%) was identified as CLABSI. The overall rate of CLABSI was (31/333) was 9.3%. The incidence density rates of HABSI and CLABSI were not significantly different between hospitals sites or patients with or without tetanus diagnosis (Table 6-2). The median time from ICU admission to diagnosis of HABSIs was 13 days (IQR 8-21 days).

Table 6-2. Incidence density rate of hospital acquired bloodstream infections

Group	HABSI rate (IQR) (per 1000 patient-days)	CLABSI rate (IQR) (per 1000 catheter-days)
All patients	3.76 (2.77-5.1) (44/11688)	8.43 (5.83-12.1) (31/3677)
Hospital		
TV	4.16 (2.12- 7.91) (10/2403)	9.51 (4.65- 18.67) (9/946)
HTD	3.52 (2.28- 5.36) (23/6539)	10.32 (5.75- 18.07) (13/1260)
NHTD	4.01 (2.11- 7.39) (11/2746)	6.12 (2.99- 12.03) (9/1471)
Tetanus diagnosis		
No tetanus	3.06 (1.97- 4.71) (22/7192)	8.06 (5- 12.81) (19/2358)
Tetanus	4.89 (3.15- 7.53) (22/4496)	9.1 (4.94- 16.31) (12/1319)

6.3.3. Aetiology of hospital acquired infections

Among the 44 patients with HABSIs, 47 isolates were identified from 44 blood cultures, including 41 patients with a single organism and 3 patients with dual organisms (1 case of co-infection with *Enterococcus faecalis* and *Proteus mirabilis*; 1 case with *Klebsiella aerogenes* and *Pseudomonas aeruginosa*, and 1 case with *A. baumannii* and *P. aeruginosa*). Of 47 isolates, 36.2% (17/47) were *Enterobacteriaceae*, 40.4% (19/47) were non-Enterobacteriaceae Gram-negative bacteria and 23.4% (11/47) were Gram-positive bacteria. The distribution of pathogens is shown in Table 6-3. The most common blood isolates were *K. pneumoniae* (11/47 or 23.4%) followed by *P. aeruginosa* (9/47 or 19.1%), *A. baumannii* and CoNS (each of 4/47 or 8.5%).

Table 6-3. Aetiology and resistance of hospital acquired infections in patients with laboratory confirmed HAIs

Pathogen	Number of isolates	Proportion (%)
Klebsiella pneumoniae	11	23.4%
Pseudomonas aeruginosa	9	19.1%
Coagulase-negative staphylococci	5	10.6%
Acinetobacter baumannii	4	8.5%
Staphylococcus aureus	4	8.5%
Proteus mirabilis	3	6.4%
Enterococcus faecalis	2	4.3%
Stenotrophomonas maltophilia	2	4.3%
Elizabethkingia meningoseptica	1	2.1%
Burkholderia seminalis	1	2.1%
Serratia marcescens	1	2.1%
Escherichia coli	1	2.1%
Klebsiella aerogenes	1	2.1%
Providencia stuartii	1	2.1%
Klebsiella oxytoca	1	2.1%

6.3.4. Risk factors for hospital acquired bloodstream infections

In univariate analysis, factors significantly associated with the occurrence of HABSI were central catheter placement (OR 2.087 95%CI 1.051, 4.142, p=0.036), arterial catheter placement (OR 2.287, 95%CI 1.264, 4.139, p=0.006) and CNS infection (OR 0.38, 95%CI 0.149, 0.967, p=0.042) (Table 6-4). In multiple logistic regression analysis, arterial catheter placement was the only independent risk factor for the occurrence of HABSI. Patients who had an arterial catheter placement showed a 3.37-fold (CI 95%: 1.483, 7.657) greater chance of developing HABSI compared with patients who had no arterial catheter placement.

The all-cause ICU-mortality rate was 189/591 (32.0%). Among 44 patients with HABSI, 16/44 (36.4%) died.

6.4. Discussion

This is the first prospective report of incidence density rates of HABSI and CLBSI, in a cohort of ventilated patients on three ICUs in Vietnam. We found a high incidence density rate of CLBSI among newly intubated patients admitted to ICUs in Vietnam with the top 3 most common pathogens being *K. pneumoniae*, *P. aeruginosa* and CoNS.

In the period of 1995-2010, the incidence rate of CLABSI in LMICs was much higher than in HICs (12.2 per 1000 catheter-days vs 3.5 per 1000 catheter-days) (World Health Organization., 2011). In high income countries, a systematic review of 4109 central lines in ICUs in the US and Korea reported an incidence rate of CLABSI from 9.8% to 20.9% (Larsen et al., 2019). In a systematic review of 200 prospective studies in adult patients with intravascular devices between January 1966 and July 2005, the incidence density rate of BSI varied by specific type of intravenous device and among short-term intravenous devices. It was lowest with peripheral intravenous catheters (0.5 per 1000 catheter-days) and highest in central venous catheters (2.7 per 1000 catheter-days) and the incidence rates were 0.1% and 4.4% respectively (Maki et al., 2006).

Table 6-4. Risk factors associated with hospital acquired bloodstream infections

Characteristics		Patients without HABSI (n = 547)	Patients with HABSI (n = 44)	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P value
Sex	Female	173 (29.3 %)	10 (1.7 %)				
	Male	374 (63.3 %)	34 (5.8 %)	1.838 (0.903, 3.741)	0.093	2.046 (0.95, 4.408)	0.068
Age	18-47 yrs	191 (32.3 %)	13 (2.2 %)				
	48-65 yrs	182 (30.8 %)	11 (1.9 %)	1.028 (0.458, 2.307)	0.947	0.782 (0.317, 1.931)	0.594
	≥66 yrs	174 (29.4 %)	20 (3.4 %)	1.473 (0.731, 2.969)	0.279	1.294 (0.529, 3.169)	0.572
Hospital	BVTV	125 (21.2 %)	10 (1.7 %)				
	HTD	271 (45.9 %)	23 (3.9 %)	0.782 (0.371, 1.649)	0.519	0.334 (0.076, 1.468)	0.147
	NHTD	151 (25.5 %)	11 (1.9 %)	0.915 (0.385, 2.176)	0.84	0.833 (0.218, 3.183)	0.789
APACHE II score	<16	198 (34.1 %)	21 (3.6 %)				
	16-20	160 (27.5 %)	9 (1.5 %)	0.59 (0.27, 1.29)	0.186	0.628 (0.24, 1.641)	0.342
	≥21	179 (30.8 %)	14 (2.4 %)	1.001 (0.507, 1.974)	0.999	1.343 (0.431, 4.184)	0.611
Charlson index	0	333 (56.3 %)	28 (4.7 %)				
	1-2	128 (21.7 %)	5 (0.8 %)	0.637 (0.246, 1.65)	0.353	0.6 (0.206, 1.742)	0.347
	3-4	38 (6.4 %)	6 (1 %)	2.324 (0.957, 5.641)	0.062	1.49 (0.403, 5.509)	0.55
	≥5	48 (8.1 %)	5 (0.8 %)	1.507 (0.581, 3.908)	0.399	1.387 (0.404, 4.755)	0.603
Sepsis and septic shock	No	412 (69.7 %)	38 (6.4 %)				
	Yes	135 (22.8 %)	6 (1 %)	0.862 (0.363, 2.05)	0.737	0.576 (0.192, 1.725)	0.324

Characteristics		Patients without HABSI (n = 547)	Patients with HABSI (n = 44)	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P value
Tetanus	No	389 (65.8 %)	22 (3.7 %)				
	Yes	158 (26.7 %)	22 (3.7 %)	1.599 (0.875, 2.924)	0.127	1.294 (0.378, 4.428)	0.681
CNS infection	No	425 (71.9 %)	39 (6.6 %)				
	Yes	122 (20.6 %)	5 (0.8 %)	0.38 (0.149, 0.967)	0.042	0.498 (0.126, 1.973)	0.321
Diabetes	No	378 (68.1 %)	34 (6.1 %)				
	Yes	133 (24 %)	10 (1.8 %)	1.157 (0.57, 2.349)	0.687	0.941 (0.373, 2.377)	0.898
Moderate and severe liver disease	No	519 (89.9 %)	42 (7.3 %)				
	Yes	14 (2.4 %)	2 (0.3 %)	2.069 (0.498, 8.589)	0.317	1.604 (0.268, 9.605)	0.605
Central catheter placement	No	247 (41.8 %)	11 (1.9 %)				
	Yes	300 (50.8 %)	33 (5.6 %)	2.087 (1.051, 4.142)	0.036	1.368 (0.628, 2.98)	0.431
Arterial catheter placement	No	402 (68 %)	22 (3.7 %)				
	Yes	145 (24.5 %)	22 (3.7 %)	2.287 (1.264, 4.139)	0.006	3.369 (1.483, 7.657)	0.004
Time from admission to ICU discharge	<12 days	205 (34.9 %)	4 (0.7 %)				
	12-25 days	180 (30.7 %)	13 (2.2 %)	0.846 (0.258, 2.768)	0.782	0.624 (0.183, 2.13)	0.452
	>25 days	158 (26.9 %)	27 (4.6 %)	0.912 (0.28, 2.971)	0.879	0.603 (0.175, 2.079)	0.423

Whilst we still found Gram negative bacteria as the most common causes of HABSI in Vietnam, Gram positive bacteria have overtaken Gram negative bacteria as the predominant cause of HABSI in the US from 1987 (Karchmer, 2000). In a large surveillance study in US hospitals between 1995 and 2002 with 24,179 cases of hospital acquired BSI, the proportion of polymicrobial infection was 13% and among monomicrobial infection, Gram-positive bacteria (65%) were predominant over Gram-negative bacteria (25%) (Wisplinghoff et al., 2004). Among 20,978 isolates HABSI in 49 US hospitals, the most common pathogens were coagulase-negative staphylococci (CoNS) (21.3%) followed by *Candida* species (10.2%) and *Enterococcus* species (9.8%). *A. baumannii* ranked 10th among the most common isolates (1.6%) (Wisplinghoff et al., 2004). In a large cohort of 5,648 patients for nearly 20 years (1995-2013) in a paediatric tertiary center in Canada, the most common cause of central venous catheter–associated bloodstream Infection was Gram-positive bacteria (72% of all CLABSI) with the predominant pathogens of CoNS (54% of all CLABSI), following by *S. aureus* (9%) and *Enterobacter sp.* (7.2%) (Carter et al., 2016).

In a surveillance of 1121 HABSI episodes in 1004 patients in Brazil, an upper middle income country, the incidence of HABSI remained unchanged at 11.2-10.2 per 1000 catheter days over 5 years with the most common pathogen being *S. aureus* (24.4%), followed by *A. baumannii* (19.2%) and CoNS (11.3%) (Girao et al., 2008).

HABSI is associated with poor outcomes, also in high income settings. In above mentioned studies, the overall mortality rate was 27% in US (Wisplinghoff et al., 2004), 38 % in Brazil. Among European countries, the overall mortality rates was lowest in France at 12% and highest in Belgium at 31.8% and in the middle for Finland (16%) and Spain (24-27.3%) (Goto and Al-Hasan, 2013). In a case control study of patients with HABSI matched by diagnosis, age, sex and length of stay before HABSI, the overall mortality rates in cases and controls was 50% and 15% respectively, corresponding to an attributable mortality of 35% (Pittet et al., 1994).

The data from LMICs was limited. In my previous retrospective study on patients with bloodstream infection in the National Hospital for Tropical Diseases from 2011 to 2013 ((Dat et al., 2017b)), I identified 77 patients with hospital acquired BSI (defined as a positive blood culture after >48h of admission). Among pre-defined patients with HABSI, the most common pathogens were *K. pneumoniae* (28.6%, followed by *E. coli* (11.7%),

Stenotrophomonas maltophilia (10.4%), *Acinetobacter* species (9.1%) and *S. aureus* (9.1%). The proportions of ESBL production in Enterobacteriaceae pathogens causing HASBI were marginally-significant higher than in community acquired BSI (4/19, 21.1% vs 5/82, 6.1%, p= 0.062) for *K. pneumoniae* and were similar for E. coli (5/9, 55.6% vs 28/52, 53.8%, p=1) (Dat et al., 2017b). However, without clinical judgment, my previous retrospective study may overestimate number of HABSI cases.

A strength of this study was that it was conducted within a multi-center randomised control trial for preventing VARI. Suspected HAI episodes were evaluated followed by standard procedures which helped to reduce the inter-hospital variability in timing and indication of diagnostic investigations. Additionally, it makes our results generalizable to intubated patients in other ICUs in Vietnam. A limitation of our study is the representativeness of our sample of intubated patients of ICU patients in Vietnam and elsewhere. As reported in a previous study, the rate of intubation among patients in critical care units in Vietnam was 52.3% (Phu et al., 2016) and tetanus patients in Vietnam are mostly treated in 2 study hospitals (NHTD and HTD) and are therefore overrepresented, which we addressed by analysing them separately.

6.5. Conclusion

Based on data of an RCT, we reported a relatively high incidence of CLABSI, risk factors for HABSI and a complex aetiology of HABSI in critical care units in Vietnam. The findings of my study suggest a necessity for national surveillance for benchmarking to measure the impact of interventions through national guidelines / prevention bundles for management of intravascular catheter-related infection.

Chapter 7: Descriptive analysis of ventilator associated respiratory infections in a randomised controlled trial in the ICUs in Vietnam

7.1. Introduction

Ventilator associated respiratory infections (VARIs) are the most common type of hospital acquired infection in ICUs worldwide, with an incidence density ranging from and 3.2 to 56.9 per 1000 ventilator days in low to middle income countries (LMIC) (Allegranzi et al., 2011). Prevention of VARI would benefit individual patients in terms of reducing the length of ICU stay, cost of care and the exposure to broad spectrum antibiotics and in turn, antimicrobial drug resistance. At institutional level, it also provides benefits to the ICUs in terms of reducing use and cost of broad-spectrum antimicrobials and reducing the exposure of environmental bacteria (such as *A. baumannii*) to these agents, which may drive down antimicrobial resistance on the units. And therefore, at national level, prevention of VARI substantially reduces morbidity and VARI-related mortality, as well as the national health budget (see Chapter 4). As I estimated in Chapter 4, efforts to reduce VARI incidence density by 1% can result in a decrease of 1,578 VARI episodes per year and a saving of US$ 1.86 million nationally.

Care bundles are a group of interventions usually consisting of 3-5 evidence-based interventions to improve patient care (Fulbrook and Mooney, 2003, Horner and Bellamy, 2012). They have gained interest for over 20 years because the clinical outcome improvement is likely a result of implementation of several different elements at the same time rather than a single intervention (Lavallee et al., 2017). Interventions incorporated into care bundles may be based on different levels of evidence, from expert opinion to proper RCTs (Camporota and Brett, 2011). As evidence is ever evolving, elements of care bundles are required to be continuously re-evaluated and updated.

Care bundles are important for VARI prevention and largely focus on the general strategies of training and education, hand hygiene, bed head elevation and early mobility, strategies to prevent aspiration and strategies to reduce colonization and contamination (Klompas et al., 2014). However, data on the effectiveness of the components of such bundles in resource constrained settings are limited and their implementation is

challenging due to inadequate staffing levels, the low rate of adherence and the findings that some interventions proven to work in developed world settings, such as semi-recumbent patient positioning, have failed to demonstrate effectiveness when scrutinized in these settings (Loan et al., 2012).

Maintenance of adequate endotracheal cuff pressure (at least 20 mmHg) is a component of VARI prevention bundles, acting in theory by diminishing the aspiration of contaminated oropharyngeal secretions (Rello et al., 1996). Utilisation of continuous control of endotracheal cuff pressure could be an ideal approach in LMIC as this intervention requires less nursing workload and has little cost in terms of disposable items (as compared to innovative endotracheal tubes, which carry a high disposable cost (Fernandez et al., 2012)). One individual patient data meta-analysis evaluated 2 randomized controlled trials (RCTs) and 1 quasi-randomised study in a high resourced settings with a total of 543 patients and found that continuous control of cuff pressure significantly reduced the incidence of ventilator associated pneumonia (VAP) (HR of 0.47, 95 % CI 0.31–0.71) (Nseir et al., 2015, Lorente et al., 2014, Nseir et al., 2011, Valencia et al., 2007). A more recent systematic meta-analysis included the 3 abovementioned studies and 4 other RCTs (1 study from France and 3 studies from China) and showed a lower incidence of VAP in the group of continuous cuff pressure control (CPC) (OR=0.39, 95% CI: 0·28–0·55) (Wen et al., 2018). However, there were no differences in the duration of mechanical ventilation, in-hospital mortality and duration of antimicrobial therapy (Wen et al., 2018, Nseir et al., 2015). Limitations of these RCTs were that only one study looked at antimicrobial prescription (Nseir et al., 2011) and additional major limitations in findings in RCTs were the heterogeneity among small sample size trials using different devices and the unblinded assessment of VAP (except in 1 trial in Spain (Lorente et al., 2014)).

Because the current evidence is insufficient to recommend the automated continuous or manual control of cuff pressure, we aimed to evaluate the impact of continuous pressure control on the incidence of VARI and the number of antibiotic-free days during ICU stay. However, although I have led the trial to completion of enrolment and follow-up, unblinded results were not available yet at the time of the deadline for submission of the thesis. Because of the time limitation for analysis, I have described here only the characteristics of patients with VARI in 4 intensive care units across 3 sites in Vietnam and a summary of the statistical analysis plan for the trial.

7.2. Methods

7.2.1. Study design

This was an open-label, randomised, controlled trial conducted at ICUs in 3 referral hospitals in Vietnam, namely the Hospital the National Hospital of Tropical Diseases (NHTD) in Hanoi and the Hospital for Tropical Diseases (HTD) and Trung Vuong Emergency Hospital (TVH) in Ho Chi Minh City. For the purpose of this study, we used a manual cuff pressure manometer (VBM Cuff Pressure Gauges, reference 54-05-000, VBM Medizintechnik GmbH, Sulz am Neckar, Germany) for the control and a stand-alone CPC device (reference 701; TRACOE medical GmbH, Nieder-Olm, Germany) for the intervention.

The inclusion criteria were patients who were at least 18 years of age, received endotracheal or tracheostomal intubation or tracheostomy for less than ≤ 24 h at the time of randomisation and for active treatment (i.e., physician caring for patient would prescribe an antibiotic if the patient developed an infection). Exclusion criteria were previous enrolment in the study or previous intubation within the last 14 days or known or suspected tracheal stenosis, tracheomalacia or stridor secondary to physical tracheal injury. The written informed consent was required to be enrolled for all participants.

The trial was registered at ClinicalTrials.gov (NCT02966392) before initiation of patients enrolment (Dat et al., 2018).

7.2.2. Sample size and randomisation

We calculated the sample size to detect a 40% reduction of VARI prevalence at the power of 80% and the significance level of 5% (two-sided test). In the setting of high burden of tetanus among patients admitted to participating ICU, we stratified the study population at a ratio of 3:7 by the admission diagnosis of tetanus and non-tetanus to generalise our study for other settings. We assumed that “lost to follow up” or “extubation within 48 hours” would account for more than 8% of participants, that the required sample size was therefore 600 patients, including 420 non-tetanus and 180 tetanus. All patients were randomly assigned in a 1:1 ratio by site and admission diagnosis to receive manual, intermittent CPC (control group) or automatic, continuous CPC (intervention group) using computer-generated block randomisation.

7.2.3. Intervention

The cuff pressure was targeted to 25 cm H_2O and recorded every 8 hours in all patients on mechanical ventilation. The control group received manual cuff pressure check and adjustment at the above schedule whilst the intervention group required no additional action. On a daily basis, the attending doctors evaluated patients until 2 days after extubation for new hospital acquired infection. When an infection was suspected, a standard panel of testing was performed with complete blood count, procalcitonin, arterial blood gas, blood culture, sputum/endotracheal aspirate microscopy and culture, urine culture and chest x-ray. Participants were followed up until discharge from ICU, and at 28 days and 90 days after randomisation (whichever happened first).

7.2.3.1. Endpoints

The primary endpoint was the prevalence of VARI (defined as VAP or ventilator-associated tracheobronchitis (VAT) during ICU stay). An independent reviewer reviewed all suspected hospital acquired infections at the end of the study and conclusively made a diagnosis of VARI. The VARI diagnosis required 2 core criteria: the first criterion was that the patient had to be on intubated for at least 48 hours and the second criterion was that new antibiotics(s) were started or the antibiotic regimen was changed to treat a new infection. When those two core criteria were met, VARI was defined as VAT if there was a new onset of purulent respiratory secretions or a change in the appearance of sputum or an increase in volume of sputum plus either a temperature >38 °C or < 36°C or a white blood cell count <4.0 x 10^9/L or ≥12 x 10^9/L with no other recognised cause. VARI was defined as VAP if a patient had a new infiltration or progressive changes on chest radiography and 2 of 3 the following criteria: (1) temperature >38 °C or < 36 °C; (2) white blood cell count <4.0 x 10^9/L or ≥12 x 10^9/L with no other recognised cause and (3) a new onset of purulent respiratory secretions or a change in appearance of sputum or increase in volume of sputum.

Secondary endpoints included microbiologically confirmed VARI and VAP, duration of intubation and mechanical ventilation in ICU, number of intubation days with antimicrobials, incidence of other HAIs, the length and cost of ICU/hospital stay, cost of antibiotics during ICU/hospital stay, in-hospital mortality and mortality at 28 and 90 days after randomisation. The definitions for secondary endpoints were detailed in our

published protocol (Dat et al., 2018). Safety endpoints included the incidence of in-hospital re-intubation and complications of intubation.

7.2.4. Statistical Analysis

Data was managed in a computerised data entry system with double data entry and analysed using R. The primary and secondary endpoints will be analysed using intention-to-treat, per-protocol and logistic regression model approaches, with an adjustment for tetanus diagnosis. For the primary endpoint patients who were intubated for less than 48 hours were excluded. The cumulative incidence of VARI was plotted by predefined subgroups, including tetanus vs. non-tetanus, with death, extubation and ICU discharge as combined competing risks. For VARI episodes, curves will be compared using log-rank test and fitted cause-specific proportional hazards model with adjustment for tetanus status as main effect. For both the logistic regression model and the cause-specific hazards regression model, I will test for heterogeneity of the intervention effect by tetanus status and report the hazard ratios. All secondary outcomes will be adjusted for tetanus status as main effect. Binary secondary endpoints (clinical and microbiologically confirmed VAP and any HAI) will be analysed in the same way as the primary endpoint. The distribution of duration of ventilation and intubation and the time of ICU stay were estimated; death will be considered as a competing event. Non-parametrical estimation will be used for the cause-specific cumulative incidence for both event types. The cause-specific cumulative incidence of death will be compared between 2 arms using the Fine-Gray competing risk regression model and will be adjusted for tetanus status. We also will fit a cause-specific proportional hazards model.

The proportion of intubated days free of antibiotics will be analysed using a Poisson regression model with the number of intubated days without antibiotics as the outcome, the randomized arm as the main covariate, and the (log-transformed) total number of intubated days as an offset. Quasi-likelihood will be used to account for potential over-dispersion. Mortality will be compared by Kaplan Meier curves and modelled by Cox proportional hazards regression. The proportion of patients with at least one adverse event will be compared using Fisher’s exact test.

7.3. Results

Five hundred ninety-seven patients were enrolled into the trial between November 2016 and December 2018. Two hundred ninety-five were allocated to the first arm and 302 patients to the second arm to receive either automated continuous cuff pressure control or manual cuff pressure control. Six patients (6/597 or 1%) were excluded from this analysis, including 2 patients who had received intubation within 14 days before study enrolment, 2 cases with wrong arm allocations, 1 patient intubated for more than 24 hours before enrolment, and 1 case with technical issues of the study device. In this chapter, a total of 591 patients were included and there were a total of 180 (%) patients with tetanus. Among 591 eligible patients recruited in the RCTs, 140/591 patients (23.7%) developed VARI, among whom 102/140 (72.9%) patients had VAP as defined by an independent endpoint reviewer. The baseline characteristics of patients were shown in

. The final analysis with unblinding of treatment arms is ongoing.

7.4. Discussion

My preliminary results showed that VARI is a common problem among intubated patients in ICU in Vietnam with the proportion of VARI in patients with mechanical ventilation (23.7%) similar to our previous report (92/374, or 24.6%) from 4 study ICUs in Vietnam from between 2013 and 2015 (Phu et al., 2017). In this prospective study, 37/92 (40.2%) patients with VARI were diagnosed with VAP and the in-hospital mortality rate was not statistically increased among patients with VAP (HR 1.79, 95% CI 0.84–3.82, p = 0.13) and VAT (HR 0.43, 95%, CI 0.13–1.38, p = 0.15) (Phu et al., 2017).

In a prospective, multicentre study in 13 ICUs from 8 European countries, 84 VARI episodes were diagnosed in 244 adult patients (34.4%), including 33 episodes of VAP (39.3%) and 51 episodes of VAT (Ramirez-Estrada et al., 2018) using the same 2008 CDC criteria as my study and the previous study in Vietnam. Participants in the European study were 63.1% male, had a median age of 56 (95%CI 49-69) and a median APACHE II score at admission of 20 (95% CI 14–27). The tracheostomy rate was 29% (44/244) and the median length of stay on ICU was 14 days (95% CI 8–26). Patients with VAP had a higher mortality than patients with VAT (42.8 vs 19.6%, p = 0.007) (Ramirez-Estrada et al., 2018).

Table 7-1. Baseline characteristics of participants

	All patients (n=591)	Patients with VARI (n=140)	Patients with VAP (n=102)
Male sex (n, %)	408/591 (69.0%)	110/140 (78.6%)	81/102 (79.4%)
Age (years), (median, IQR)	58.0 (42.0-70.0)	58.0 (39.8-68.0)	59.0 (39.2-69.0)
Place of enrolment (n,%)			
TVH	135/591 (22.8%)	11/140 (7.9%)	9/102 (8.8%)
HCM	294/591 (49.7%)	83/140 (59.3%)	62/102 (60.8%)
NHTD	162/591 (27.4%)	46/140 (32.9%)	31/102 (30.4%)
Transferred from other hospital (n,%)	422/591 (71.4%)	111/140 (79.3%)	75/102 (73.5%)
Initial intubation			
endotracheal intubation	438/591 (74.1%)	83/140 (59.3%)	53/102 (52.0%)
tracheostomy	153/591 (25.9%)	57/140 (40.7%)	49/102 (48.0%)
APACHE II score at enrolment (median, IQR)	17.0 (13.0-22.0)	15.5 (13.0-19.0)	14.0 (12.0-19.0)
Charlson Comorbidity Index			
0	361/591 (61.1%)	111/140 (79.3%)	80/102 (78.4%)
1-2	133/591 (22.5%)	19/140 (13.6%)	14/102 (13.7%)
3-4	44/591 (7.4%)	6/140 (4.3%)	5/102 (4.9%)
≥5	53/591 (9.0%)	4/140 (2.9%)	3/102 (2.9%)
Cause of admission (n,%)			
Tetanus	180/591 (30.5%)	70/140 (50.0%)	59/102 (57.8%)
Pneumonia (any)	167/591 (28.3%)	22/140 (15.7%)	15/102 (14.7%)
Sepsis/septic shock	141/591 (23.9%)	16/140 (11.4%)	11/102 (10.8%)
CNS Infection	127/591 (21.5%)	33/140 (23.6%)	18/102 (17.6%)
COPD	10/591 (1.7%)	0/140 (0.0%)	0/102 (0.0%)
Cerebrovascular disease	9/591 (1.5%)	1/140 (0.7%)	1/102 (1.0%)
Myocardial infarction	27/586 (4.6%)	2/140 (1.4%)	2/102 (2.0%)
Others	66/591 (11.2%)	11/140 (7.9%)	8/102 (7.8%)

Characteristics of patients admitted to ICUs were similar previous reports, in terms of age, sex distribution and severity. Characteristics of patients admitted to ICUs are recognised as a non-modifiable risk factor for the development of VAP and for mortality (Bonten et al., 2004, Blot et al., 2014). These patients related factors included comorbidities (chronic diseases, head trauma, coma, organ failure), and age > 60 years old (Bonten et al., 2004). As I have discussed in 1.2.1.Overview of hospital acquired infections (HAIs) and Figure 1-3, VAT and VAP are considered a continuum of ventilator-associated respiratory tract infections and the differentiation of these 2 entities is challenging. A recent review hypothesised that the continuum and severity of VARI was modulated by host immunity, inflammation and causative pathogens (Wu et al., 2019). In patients with intact immunity, VARI can be limited to local inflammation as VAT whilst patients with immunosuppression are at higher risk of VAP development. Therefore, the information of patient characteristics was important for generalising findings to other settings and better understanding the risk of HAIs acquisition.

To my best knowledge, this trial is the first study with a large sample size in an LMIC to evaluate the effectiveness of endotracheal cuff CPC in preventing VARI. Because of the limitation of human resources in LMICs, besides reducing the nursing workload for maintaining cuff pressure, the intervention should show a beneficial impact on patients outcomes, e.g. reducing mortality, duration of mechanical ventilation and ICU stay, cost of admission etc. The intervention will likely be considered of questionable benefit should it result only in a reduction in VARI episodes without corresponding reductions in at least patient-centred or antibiotic stewardship outcomes (Barlam et al., 2016).

The drop of cuff pressure in intermittent measurement can lead to leakage of secretions through the endotracheal tube cuff (Carter et al., 2014). Current available evidence in small, non-RCT studies are insufficient to recommend CPC routinely (Tablan et al., 2004). My study provides an opportunity to evaluate the impact of CPC in preventing VARI and in reducing antibiotic use as an aspect of infection prevention and control and antimicrobial stewardship in a resource-constrained setting with established high levels of antibiotic use and resistance.

Both VAT and VAP share common clinical manifestations of fever, purulent respiratory secretions, and leucocytosis. The chest x-ray is the usual common method to diagnose VAP based on the presence of pulmonary infiltrates. In a prospective study in 10 countries with

114 ICUs, the incidence of VAT was similar to VAP (11% vs 12%) (Martin-Loeches et al., 2015). In a survey of perceptions of international physicians, nearly 80% of respondents diagnosed VAT on the basis of both clinical and microbiological criteria, and the remaining respondents used clinical criteria alone or as a diagnosis of exclusion. Half of the respondents agreed to antibiotic use in patients with VAT (Rodriguez et al., 2014). In Vietnam it is common practice to treat VAT with antimicrobials, and in view of these considerations, we constructed our trial around an endpoint of VARI that encompassed both VAT and VAP.

In an open-label study where the treating doctor is not blinded to the intervention, there is a risk of clinical ascertainment bias of the study endpoint (Schulz and Grimes, 2002). Whilst death or microbiological results are hard endpoints which are unlikely to be biased by a lack of blinding, VARI diagnostic categories and antibiotic use are more subjective and vulnerable to misclassification bias in non-blinded trials (Dechartres et al., 2009). For this reason an independent endpoint committee is recommended for multicentre clinical trials to judge complex, multi-aspect definitions and outcomes (Naslund et al., 1999, Moher et al., 2001). In our study treating doctors know the allocated arm of the patient and perform assessments for HAI as part of routine care; therefore, the use of a blinded endpoint reviewer will reduce bias in the primary endpoint evaluation. The study may also offer an opportunity to understand inconsistencies in the assessment of VARI diagnoses between treating doctors and the endpoint reviewer, as well as how this impacts the significance of results.

7.5. Conclusion

The rate of VARI in my study was high as 23.7% and similar to previous study in Vietnam in the period of 2013-2015. The final analysis for the effectiveness of continuous cuff pressure control vs manual cuff pressure control is ongoing. Patient recruitment and follow up is completed with a low rate of withdrawal, ensuring the validity of clinical data and the acceptability for participants.

Chapter 8: **Discussion**

Vietnam has made substantial progress over the past decade regarding the growth of the healthcare workforce, but the national capacity of critical care was still remarkably lower than in other countries in Southeast Asia and other low- and middle-income regions (Dat et al., 2017a). Implementation of infection prevention and control and antimicrobial stewardship in Vietnam, like in other LMICs, is limited by the capacity of healthcare infrastructures, microbiological diagnostics, effective interventions, political action and insufficient financial resources (Cox et al., 2017, Nguyen et al., 2013).

Because the understanding of antimicrobial use is important when addressing antimicrobial drug resistance whilst the data of antimicrobial consumption in Vietnam was dispersed in different sources, my thesis started by analysing the availability and use of antimicrobials in the country. My findings in Chapter 3 and Chapter 4 showed that the high drug resistance rate in Vietnam required the use of last resort high-price antimicrobials and it was associated with high cost of treatment. Under the current the economic situation, the prevention of hospital acquired infections (HAIs) is of importance to the country in terms of saving health expenditures and improving the affordability of ICU services. Results presented in Chapter 4,Chapter 6 and Chapter 7 confirmed that ventilator associated respiratory infections (VARI) and central line-associated BSI (CLABSI) were important hospital acquired infections in intubated patients in critical care units in Vietnam. To address the burden of HAIs, my thesis aimed to explore the potential interventions which have the potential to reduce antimicrobial use, focusing on diagnostics and medical devices in 2 randomised control trials. The key messages of my thesis were further discussed below.

8.1. Hospital acquired infections are pressing on critical care services in Vietnam

The cost of antimicrobials relative to total medication and healthcare cost in Vietnam was high in comparison to high income settings (Chapter 4), especially the antimicrobials of last resort. Currently, the medication budget of public hospitals is spent for a substantial proportion on antimicrobials (28.5% of hospital budget), which is higher than high income countries (nationally 9% of a hospital's drug acquisition budget in Canada (Nault et al., 2008)).

Because hospital acquired infections result in additional use of resources, including antimicrobial therapy and diagnostics, policy decisions on infection prevention and control and antimicrobial stewardship, including conserving antibiotic effectiveness and accessibility and utilising microbial diagnostics need to be supported by evidence in a resource limited setting.

My thesis is the first work presenting the high economic burden of VARI, the most common HAIs in critical care units in Vietnam. Based on available data and resources, we carried out a modelling study to estimate the cost of management of VARI throughout Vietnam. At the time of writing this thesis, the cost of VARI to the national healthcare system in Vietnam was unavailable and therefore the magnitude of the economic burden of HAIs was lacking. My study showed the cost spent for treatment of each VARI episode was US$ 1,174.9 per episode, including US$ 600 for antimicrobials, US$ 268.2 for ventilation support, US$ 262.5 for hospital bed and US$ 43.9 for diagnostics. At the country level and current VARI incidence of 21.7/1000 ventilation days, the annual total lost cost for management of VARI was calculated at US$ 40,447,469. Whilst the VARI incidence was quite consistent across studies in Vietnam, the data on the incidence of BSI in critical care units is limited. Using data from my intervention trial I also report as the first study in Vietnam the incidence rate of BSI in (ventilated) patients in 3 ICUs in the country. The high rate and incidence of CLABSI (9.3% and 8.43 per 1000 catheter-days, respectively) confirmed the burden of HAIs in critical care units and the importance to implement surveillance and interventions for different HAIs types.

The same methods used in Chapter 4 can be applied to estimate the excess cost of management of other HAIs, when an alternative approach using the detailed cost of individuals to modelling the total cost or an analysis controlling for potentially confounders (e.g. age, sex, or number of co-morbidities or admission type) is not available. Regarding the methods presented in the Chapter 6, for LMICs, when national active surveillance is not established, the reports of HAIs incidence from prospective clinical trials as these can be used to inform decision making and allocating local resources to infection control programmes. Currently, multiple-partner efforts are being made in Vietnam to track HABSI and hospital acquired urinary tract infections. It is considered the first sentinel surveillance in country conducted in 6 model hospitals (Centers for Disease Control and Prevention (CDC), 2019) .

The results of HAIs burden presented in my thesis have important implications for policy and medical practice. The estimate of the economic burden of VARI and the incidence rate of BSI is evidence for the urgency of implementation of care bundles for HAIs prevention nationwide and indicates potential financial benefits of these preventions.

8.2. Advanced technology alone is not effective without human action

Among advanced microbiological diagnostics, we chose MALDITOF-MS, which is a relatively recent innovation with advantages of early identification of pathogens (Chapter 5). We searched PubMed between October 1, 2007, and October 1, 2018 with the terms “time-of-flight mass spectrometry" or “MALDI-TOF” combined with "identification" and "patients". There were 2 systematic reviews assessing MALDI-TOF performance, in terms of microbiological diagnostics accuracy in bloodstream infection by comparison between MALDI-TOF and routine microbiological methods (Scott et al., 2016, Dixon et al., 2015). There were no randomised controlled trials when we started our study. All previous clinical studies of MALDI-TOF were conducted in HIC settings. My study was the first to explore the impact of MALDI-TOF diagnostics alone on antimicrobial use and clinical outcomes in an LMIC, where antimicrobial resistance and antibiotic consumption are high. Our findings showed that advanced diagnostics alone did not improve antimicrobial use. The impact of transferring novel techniques to LMICs may need to be evaluated by the highest quality of evidence. The communication and multidisciplinary teamwork in this setting should be improved by training and education and further explored in clinical trials. Research on antimicrobial stewardship is advocated to evaluate its effectiveness on healthcare provider and patient perspectives as well as microbiological endpoints (McGregor and Furuno, 2014). Antimicrobial stewardship programs often include multiple simultaneous interventions (“a bundle”) which leads to difficulty in measuring the effect of individual relevant components. Recently, rapid diagnostics, including MALDITOF, in combination with active reporting systems have been advocated for antimicrobial stewardship to optimise antimicrobial therapy and improving clinical outcomes (Barlam et al., 2016). However, our study and a small number of other studies on other rapid diagnostic technologies indicate that the process of reporting results is likely to be as important as the speed with which results can be generated.

Chapter 5 showed that the early identification of pathogens from culture of blood and other sterile sites using MALDI-TOF, in the absence of any concurrent antimicrobial stewardship programme, did not result in a difference in the proportion of patients on optimal antimicrobial therapy within 24 or 48 hours of growth of the first clinically significant isolate, the time taken to provide optimal therapy, duration or total antibiotic therapy, outcome or time in intensive care. We did find a small improvement in the rate of hospital discharge, however in the context of other results and the impact of the exclusion of outliers on this result, it is unlikely to be of clinical significance.

In common with most studies, we found a significant shortening of the time from specimen growth to identification and reporting. Our study gives some hints as to why this did not result in an improvement in optimal therapy. The most common cause of suboptimal therapy at 24 hours was use of excessively broad therapies, suggesting that there were delays in de-escalation of therapy. Analysis of the rationale for failure to de-escalate found that most clinicians felt that the broad therapy was adequate and wished to review antibiotic susceptibility results before making decisions on therapy.

To support Vietnam to address the AMR burden, country and regional grants from the Fleming Fund, a global investment of £265 million in 24 LMICs from 2015 to 2021, totalling around £15 million, have been made available for Vietnam to establish a National AMR reference laboratory and a National AMR hospital surveillance network (Kinh et al., 2017) and for building capacity for laboratory diagnostics for AMR in hospital and veterinary laboratories. The findings of the MALDITOF-MS study implicate that the laboratory capacity for microbiological diagnosis needs to be sustained by trained human resources. Antimicrobial stewardship in participating hospitals should be implemented prior to or alongside the equipment investments.

8.3. Evidence based intervention is required for adaptation of bundle of care in prevention of HAIs in resource limited settings

In Chapter 7, I presented the methods used to evaluate the effectiveness of a medical device on antimicrobial use and HAI incidence. By focusing on VARI, which is the most common HAI in critical care units in Vietnam, my work evaluated the effectiveness of continuous cuff pressure control in preventing VARI. At the time of thesis submission, this was the largest study with an RCT design and the first study in an LMIC setting to evaluate

this method. Because of the deadline for thesis submission, the results of this study could not yet be presented here and final results will likely become available prior to the viva and will subsequently be published in an open access, peer reviewed journal.

8.4. Recommendations

Whilst the global health expenditure grows faster than GDP (World Health Organization, 2017b), our findings in the costing study illustrate the significance of HAIs prevention in saving national cost. In consideration of the findings of our MALDITOF-MS trial, studies to assess the implementation of advanced technology and medical devices are an important part of the process to improve patient care and to determine whether such investments are cost-effective in supporting antimicrobial stewardship interventions and prevention and control of HAIs. Results of these studies provide evidence to help guide policy decisions on prioritising investments for preventing HAIs and promoting antimicrobial stewardship with limited budgets.

Based on findings in my thesis, I recommend further studies to be performed to (1) evaluate the economic benefit of MALDITOF-MS as part of an antimicrobial stewardship intervention in reducing antimicrobial use; (2) measure the incidence and economic burden of different HAIs types as part of a national surveillance programme and (3) evaluate the roll-out of the continuous cuff pressure control devices to reduce HAI in resource limited settings (pending results of our trial).

Future studies of medical devices should evaluate the impact of successful interventions at the ICU level and benefits to patient's outcomes with the hope that a substantial reduction in the use of antibiotics will in turn lead to a reduction of the emergence and transmission of resistance in the ICU. Because RCTs are time and cost consuming, alternative approaches (cluster-randomised or stepped-wedge design) may be considered for any potential rollout of a successful intervention.

8.5. Conclusions

My thesis showed that whilst the burden of HCAI is severe and the high cost of treatment for multidrug resistant infections placed an economic burden on health systems and both served as a barrier to the uptake of ICU services, advanced diagnostic technology alone was not a solution for reducing antimicrobial use or improving patient's clinical

outcomes. Evaluation of interventions in clinical trials is important to understand how well technology and devices works in reality of clinical settings.

My thesis highlighted the necessity of addressing the burden of hospital acquired infections in Vietnam, where the incidence of HAIs is high but technology, financial and human resources are inadequate. The described approach to evaluate the cost of care in this thesis can be applied to explore the economic burden of anatomical site-specific HAIs types, while national registration is not established. Understanding the effectiveness stand-alone implementation of advanced diagnostic technology (MALDITOF) and the HAI prevention recommendations that will follow from my clinical trial will have wide applicability in other LMICs.

The findings in my thesis are relevant to healthcare professionals and policy stakeholders. It demonstrates the magnitude of HAI burden and creates awareness of potential beneficial interventions. Results of my trials will be helpful to inform decisions to establish the antimicrobial stewardship programmes and infection prevention and control bundles to improve patients' outcomes.

Appendix

Appendix 1: Supplementary review data for the Chapter 5

Supplementary Table 1. Review of identification of pathogens from blood culture using MALDITOF-MS

	MALDI-TOF System	Samples	Accuracy	Limitation	Ref
Schubert et al	MALDI Sensityper kit (Bruker Daltonik)	500 positive BC	86.5% of all, 89.8% of GNB, 86.3% GPB, 70.6% of yeasts at the species level	*Streptococcus mitis* isolates have been misidentified as *Streptococcus pneumoniae*	(Schubert et al., 2011)
Jo et al	MALDI-TOF Vitek MS (bioMérieux, France)	254 blood cultures: 235 from adult patients, and 19 from pediatric patients	81.8% (208/254) of all, 73.9% of GPB and 92.6% of GNB. Correct identification for *Enterobacteriaceae*, non-fermentative GNB, and staphylococci was 81/83 (97.6%), 12/15 (80.0%), and 72/85 (84.7%), respectively.	45 isolates was not identified, included 37 Gram-positive isolates (14 streptococci, 11 coagulase-negative staphylococci (CoNS), three enterococci, 2 *Staphylococcus aureus*, 2 *Micrococcus* spp., 2 *Bacillus* spp., and one each of *Actinomyces odontolyticus*, *Finegoldia magna*, and *Peptostreptococcus* spp) and 8 pediatric bottles (2 *Staph capitis*, 2 *Staphylococcus epidermidis*, and one each of *Streptococcus pneumoniae*, *Micrococcus* spp., *Streptococcus constellatus*, and *Burkholderia cepacia*).	(Jo et al., 2016)

	MALDI-TOF System	Samples	Accuracy	Limitation	Ref
Chen et al	Bruker Microflex LT with Biotyper 3.0 system	202 positive blood cultures, included 181 monomicrobial, (75 of GPB and 106 of GNB) and 21 polymicrobial.	97.8% (177/181) of monomicrobial, 23.8% (5/21) of mixed-culture specimens and only the major composition of the remaining 16 mixed cultures	*Streptococcus anginosus* was miss-identified as *Streptococcus constellatus* and *Streptococcus bovis* identified as *Streptococcus gallolyticus*. Neither system was ready for direct use with polymicrobial blood culture	(Chen et al., 2013)
	bioMérieux Vitek MS IVD		92.3% (67/181) of monomicrobial, only the major composition of mixed cultures		
La Scola et al	MALDI-TOF MTP 384 target plate (Bruker Daltonik GmbH, Leipzig, Germany	584 positive blood cultures, 562 monomicrobial and 22 polymicrobial.	Protocol 1: 59% (189/322) of all, 94% (117/125) of GNB and 37 (72/192) of GPB. Protocol 2: 76% (181/240) of all, 87% (87/100) of GNB and 67% (94/140) of GPB	*Streptococcus spp*. were poorly identified. *Serratia marcescens* was misidentified as *Aeromonas hydrophila*. 2/22 polymicrobial positive blood was not identified, 18 with only one of the two isolates was identified, 2 specimens (including one with three different species) was erroneous identification	(La Scola and Raoult, 2009)
Pulcrano et al.	Vitek II system (bioMérieux, Marcy L'Étoile, France)	82 blood culture of Candida non-albicans	99% (74/82)	*Candida parapsilosis* was misidentified as *Lodderomyces elongisporus* and *Candida tropicalis* as *Saccharomyces cerevisiae*. misidentifying most of *Candida guillermondii* isolates	(Pulcrano et al., 2013)

	MALDI-TOF System	Samples	Accuracy	Limitation	Ref
Barnini et al.	MALDI-TOF (Bruker Daltonics, Bremen, Germany)	133 positive Gram-negative bacteria blood cultures, 118 monomicrobial and 15 polymicrobial	99.2% (117/118) of GNB monomicrobial, 86.6 % (13/15) polymicrobial was correctly identified 90% (70/82) of monomicrobial GPB, 7/7 positive GPB mixtures with one microorganism of the mixture was correctly identified	*Stenotrophomonas maltophilia* strain was not identified. All microorganisms of 2 mixture cultures were unidentified. Unidentified strains were 3 *Staphylococcus epidermidis*, 1 *Staphylococcus haemolyticus*, and 1 *Micrococcus luteus*, 3 misidentified strains by the direct method were: 1 *S. haemolyticus* erroneously identified as *Staphylococcus hominis*, 1 *E. faecium* as *Enterococcus faecalis*, and 1 *Streptococcus oralis* group mitis as *Streptococcus pneumoniae*.	(Barnini et al., 2015)

GPB, Gram-positive bacteria

Supplementary Table 2. Review of effect of rapid identification of pathogens using MALDI-TOF on clinical outcome

Study design	Study population	Intervention	Outcome	Mortality	Length of stay	Ref
Retrospective, quasi-experimental single-center study	119 patients with A. baumannii pneumonia and/or bacteremia. 66 patients in pre-intervention arm and 53 patients in intervention arm	MALDI-TOF and antibiotic stewardship	19% increase in clinical cure (15% vs. 34%, p=0.016)	No significant difference in 14-day mortality (20% vs. 25%, p=0.526).	Decreased infection-related length of stay (13 [8-18] vs. 11 [7-15] days, p=0.021).	(Wenzler et al., 2016)

Study design	Study population	Intervention	Outcome	Mortality	Length of stay	Ref
Retrospective study	155 episodes of peritoneal dialysis-related peritonitis, 98 isolates using a conventional method and 57 isolates using the MALDI-TOF MS	MALDI-TOF	No difference in improvement of peritonitis, (the time to WBC < 100/mm3)	No significant difference in in hospital mortality (4.4 vs. 10%, p= 0.52)	Decreased hospital stay (8.2 ± 4.5 versus 5.2 ± 4.8 days, P = 0.001)	(Lin et al., 2016)
quasi-experimental single-center study	317 with Gram-negative bloodstream infections, final analysis included 112 patients in the pre-intervention arm and 107 patients in the intervention arm	MALDI-TOF and antibiotic stewardship	The time to an active agent initiation was shorter than in intervention group (n=5, 36.5 hours vs n=22, 73.2 hours, P <0.001)	No significant difference in all-cause 30-day mortality rates (5.6% versus 10.7%; P=0.19)	No significant difference in ICU length of stay (6.1 vs. 4.9). The mean hospital length of stay was 11.9 versus 9.3 days in the intervention group (n= 101; P <0.01)	(Perez et al., 2013)
A pre–post quasi-experimental study	501 patients with bacteremia or candidemia included in final analysis	MALDI-TOF and antibiotic stewardship	Improved time to effective antibiotic therapy (30.1 vs 20.4 hours) and optimal antibiotic therapy (90.3 vs 47.3 hours)	30-day all-cause mortality preintervention 20.3% versus with MALDI-TOF 12.7% (p=0.021)	Length of hospitalization 14.2 ± 20.6 days versus 11.4 ± 12.9 days. Length of ICU stay was 14.9 ± 24.2 days versus 8.3 ± 9.0 day	(Huang et al., 2013)
A pre–post quasi-	153 patients with antibiotic-resistant Gram-negative bacteremia hospitalised and	MALDI-TOF and antibiotic stewardship	Improved time to optimal antibiotic therapy (80.9 versus	Mortality among patients during the	Intervention decreased duration of hospitalization	(Perez et al., 2014)

Study design	Study population	Intervention	Outcome	Mortality	Length of stay	Ref
experimental study	112 patients treated post-implementation.		23.2 h) and effective antibiotic therapy (89.7 h versus 32 h)	intervention period was lower (21% versus 8.9%, P < 0.01)	(15.3 days vs 23.3 days, P < 0.0001) and shorter ICU length of stay (10.7 vs 16 days, P<Z 0.008).	

Appendix 2: Case report form – MALDI-TOF trial

Appendix 3: Case report form – VARI trial

Appendix 4: Published papers

- **Dat VQ**, Nadjm B, Campbell JI, Dung VTV, Torre A, Tu NTC, Van NTT, Trinh DT, Lan NPH, Trung NV, Hang NTT, Hoi LT, Baker S, Wolbers M, Chau NVV, Van Kinh N, Thwaites GE, van Doorn HR, Wertheim HFL. 2019. A randomised controlled trial of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) versus conventional microbiological methods for identifying pathogens: Impact on optimal antimicrobial therapy of invasive bacterial and fungal infections in Vietnam. J Infect doi:10.1016/j.jinf.2019.03.010.

- **Dat VQ**, Geskus RB, Wolbers M, Loan HT, Yen LM, Binh NT, Chien LT, Mai NTH, Phu NH, Lan NPH, Hao NV, Long HB, Thuy TP, Kinh NV, Trung NV, Phu VD, Cap NT, Trinh DT, Campbell J, Kestelyn E, Wertheim HFL, Wyncoll D, Thwaites GE, van Doorn HR, Thwaites CL, Nadjm B. 2018. Continuous versus intermittent endotracheal cuff pressure control for the prevention of ventilator-associated respiratory infections in Vietnam: study protocol for a randomised controlled trial. Trials 19:217

- **Dat VQ**, Huong VTL, Turner HC, Thwaites L, van Doorn HR, Nadjm B. 2018. Excess direct hospital cost of treating adult patients with ventilator associated respiratory infection (VARI) in Vietnam. PLoS One 13:e0206760

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